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VOL. III. ST. LOUIS, APRIL, 1918 NO. 7

ORIGINAL ARTICLES

AN INVESTIGATION OF CERTAIN PHENOMENA OF ALLERGY WITH SPECIAL REFERENCE TO THE RESPIRATORY AND CIRCULATORY SYSTEMS IN RELATION TO THE CAUSE OF DEATH*

BY MORT D. PELZ AND D. E. JACKSON, PH.D., M.D., ST. LOUIS, MO.

THE cause of death in anaphylactic shock has been supposed to vary in different species of animals, and the associated phenomena have been held to be widely dissimilar. In the sensitized guinea pig a fatal bronchoconstriction is believed to be produced by the provocative injection of the protein, while in dogs most observers have considered the cause of death to be immediately associated with the vascular derangement and weakness. Bronchoconstriction has been thought to be almost, if not entirely, absent in these animals, and aside from the changes in the vasomotor apparatus, pathologic developments in the liver, intestines, heart, etc., have been noted by Richet,[1] Biedl and Kraus,[2] Pearce and Eisenbrey,[3] Edmunds,[4] Robinson and Auer,[5] Schultz,[6] Weil,[7] Manwaring,[8] Dale,[9] Simonds,[10] Voegtlin and Bernheim,[11] and others.

In the present series of experiments we have used dogs sensitized by three injections of the protein, injected subcutaneously at intervals of about five days. At each injection six to ten cubic centimeters of the protein were used, either normal horse serum† or egg albumen being given to produce the sensitization. After twenty-one or more days the animals were etherized, pithed, and prepared for recording carotid blood pressure. Then arrangements were made for recording the contractions or relaxations of the bronchioles by a method which was described in detail by one of us (D. E. J.)[12] some years ago. For this latter purpose the special piece of apparatus devised, when fitted into the

*From the Department of Pharmacology of Washington University Medical School, St. Louis, Mo.

A preliminary report of this work was given before the American Pharmacological Society on Dec. 29, 1917, at Minneapolis, Minn.

†We have used normal horse serum with preservative sold by Parke, Davis & Co. Presumably the preservative is tricresol.

thorax through a median incision in the sterum, holds the chest wall rigidly distended, and keeps the chest cavity practically air-tight. The thoracic cavity is thus shut off from the external air except by communication through two tubes, one of which serves as an adjustable by-pass and permits air to enter the chest, while through the other, air is intermittently aspirated from the thorax. Inspiration occurs by air entering the lungs through the trachea during the forcible aspirations. Between aspirations the lungs collapse from their own elasticity (expiration) or by a contraction of the bronchioles. The pressure and rate of aspiration are kept constant and regular, about thirty per minute. The amount of air entering or leaving the lungs is recorded by means of a tambour connected with the side tube of the tracheal cannula, and writing upon a smoked drum. The extent of the down stroke of the writing lever indicates the relative amount of air entering the lungs in inspiration, the up stroke, the amount leaving in expiration. Contraction of the bronchioles decreases the amount of air entering and leaving the lungs, and thus the amplitude of the record on the drum is lessened. With the dilatation of the bronchioles the amplitude of the tracing is increased, because a relatively larger amount of air passes through the trachea at each inspiration or expiration.

Usually the brain and medulla were destroyed by pithing. For the provocative or final injection of protein quantities varying from eight up to seventeen cubic centimeters were quickly injected into the external jugular vein by means of a large syringe.

Fig. 1 shows the anaphylactic response produced immediately after the injection of the antigen. Together with the marked drop in blood pressure, there results an intense bronchoconstriction which is partially relaxed by the injection of one cubic centimeter of adrenaline (1:10000). This substance frequently saves the animals if injected *early;* that is, before the bronchial spasm has reached its greatest intensity. After the spasm is thoroughly developed, no drug so far tried will cause dilatation of the bronchioles. Forcibly dilating the lungs for several respiratory cycles, does not cause the lungs to remain relaxed when the original aspiration pressure is resumed, as would occur after almost any of the bronchoconstricting drugs. This shows how firmly the bronchioles are constricted. After the anaphylactic shock is well advanced and the asphyxia has become intense, adrenaline is no longer, or only feebly, active. We do not believe as Simonds has suggested that the decreased activity of adrenaline during the deepest part of the anaphylactic shock may be due to some specific change in the irritability of the vasomotor ganglia or medullary centers. We however, have supposed this decreased response to adrenaline to be due to the extreme grade of asphyxia present, and not to any specific action of the anaphylactic process, for practically the same bronchoconstricting phenomenon is seen after injections of a number of the opium alkaloids, and in the latter case adrenaline behaves similarly. This tracing also shows the failure of atropine to relieve the constriction of the bronchioles after the production of the shock. Several minutes later sodium nitrite was given intravenously to this animal, and it also failed to produce a relaxation.

Fig. 2 shows the action produced by injection of the protein into a sensitized dog which had received atropine just prior to the provocative injection. Enough

atropine (1¼ mg.) had been given to prevent electrical stimulation of the vagus nerve from slowing the heart; yet this did not prevent the anaphylactic phenomena. After partial recovery from the shock this dog received five cubic centimeters of codeine, and a typical normal response for this drug was ob-

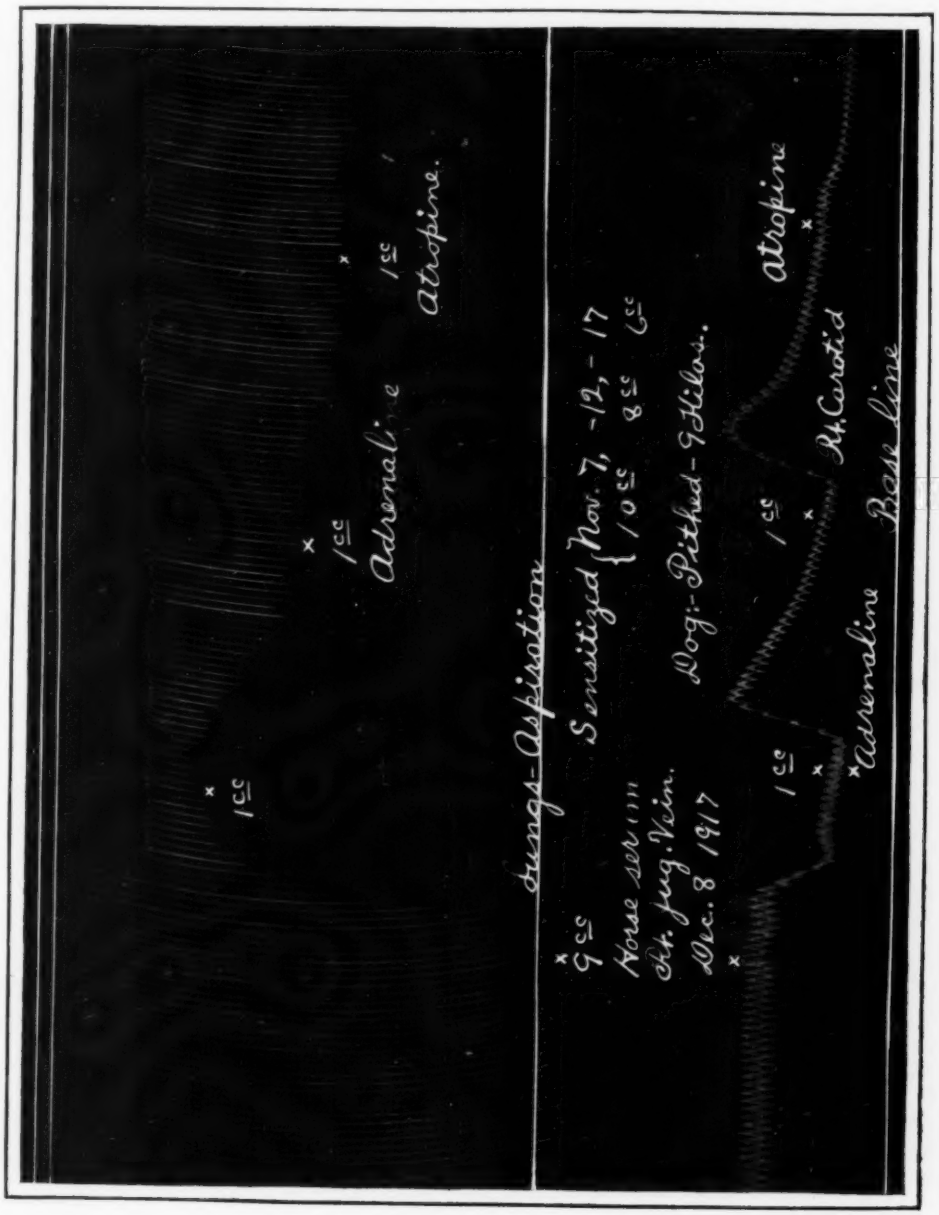

Fig. 1.—(For description, see text.)

tained (Fig. 3). As mentioned before, the action of codeine, heroine, etc., in dogs more closely resembles the anaphylactic phenomena than any substances with which we are acquainted. This similarity applies to both the bronchial

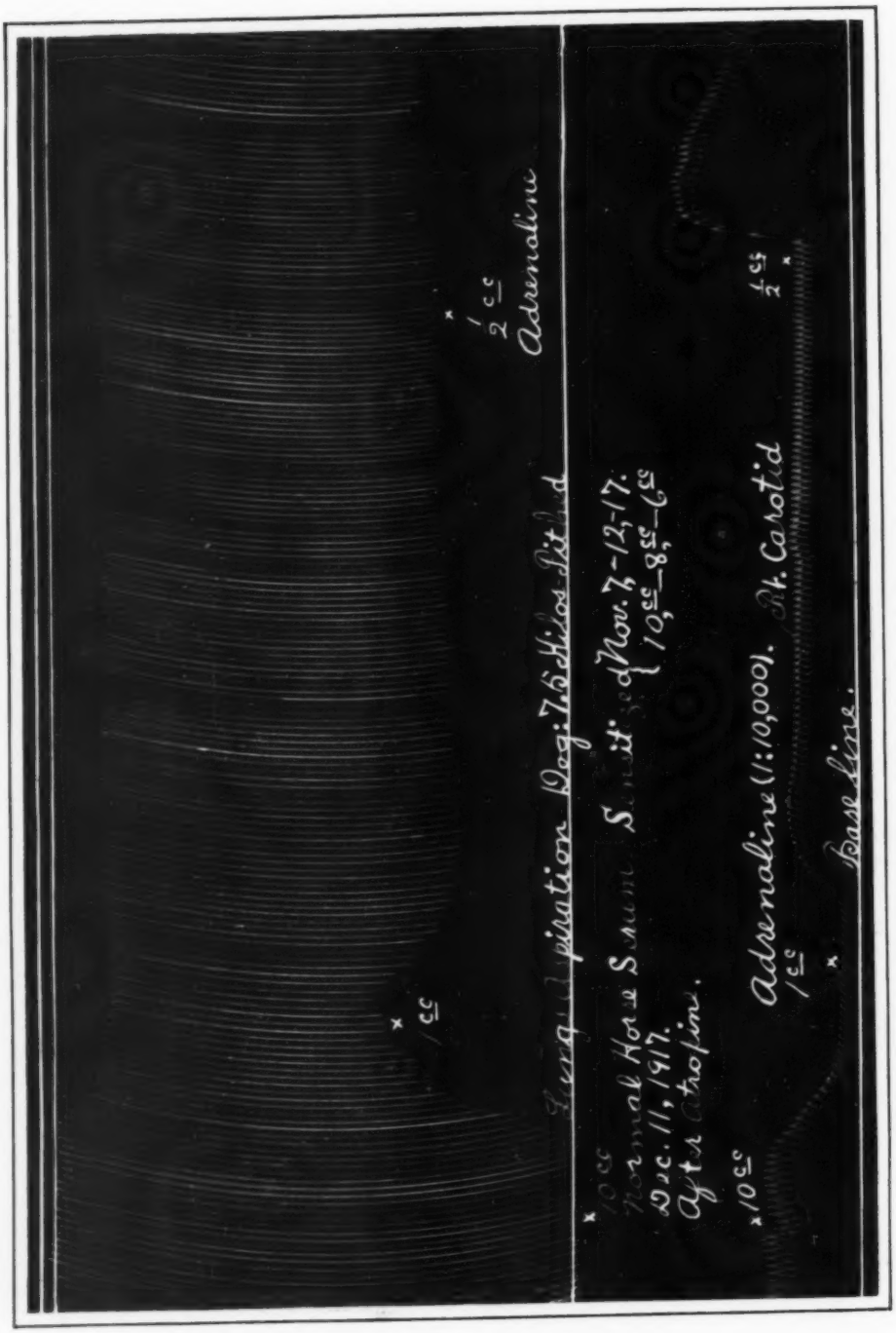

Fig. 2.

and blood pressure changes. Another point of similarity is that the action of the opium alkaloids in full doses is usually maximal, and after the animal recovers another typical response from the same substance can not be produced.

This resembles the maximal and single response produced by proteins injected into sensitized animals.

Again in the same dog after partial recovery ergamine (histamine) was given, which is shown to be active to practically its normal extent (Fig. 4). This drug has been thought by some observers to be similar (if not identical) to the substance liberated in the body during the anaphylactic shock and which produces the marked effects that occur. This is hardly probable when we consider that with a maximal dose anaphylaxis occurs but once, while the action of

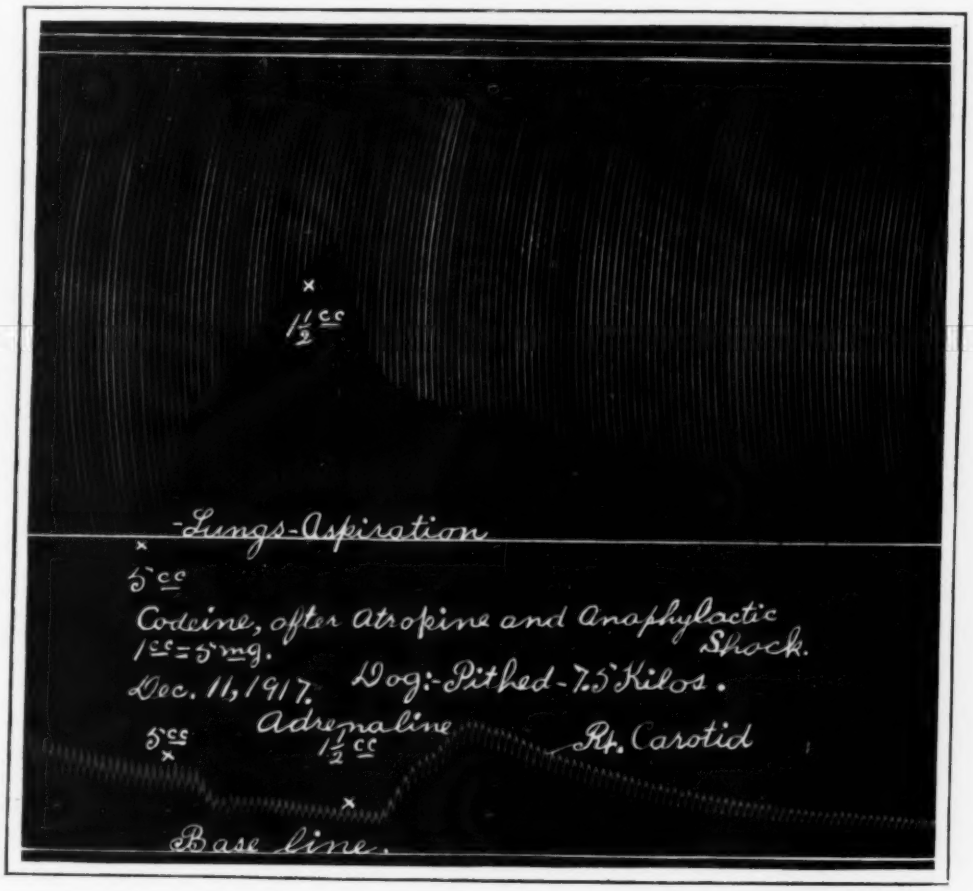

Fig. 3.

ergamine can be repeated several times, and even after partial recovery from the anaphylactic shock itself.

Fig. 5 shows the fatal bronchoconstriction in a well sensitized dog upon the injection of horse serum. Adrenaline apparently given fairly early after the production of the reaction could not cause recovery; due probably to the extreme grade of asphyxia already produced. The profound response in this animal was obtained after injections of pituitrin, and it is therefore apparent that there is no decreased susceptibility of a sensitized dog after injections of this substance.

In Fig. 6 is shown an especially marked response in an animal which was

sensitized to egg albumen. The bronchioles constricted so tightly and held on with such great tenacity that in a short time a fatal asphyxia was produced, and the production of bronchial relaxation by the administration of drugs was impossible.

The experiments of Manwaring,[8] Voegtlin and Bernheim,[11] Deneke,[13] and more recently of Richard Weil[7] have pointed towards the liver as essential to the vasomotor depressions and other changes which they have recognized as occurring in anaphylactic shock. In support of this view Weil found that the

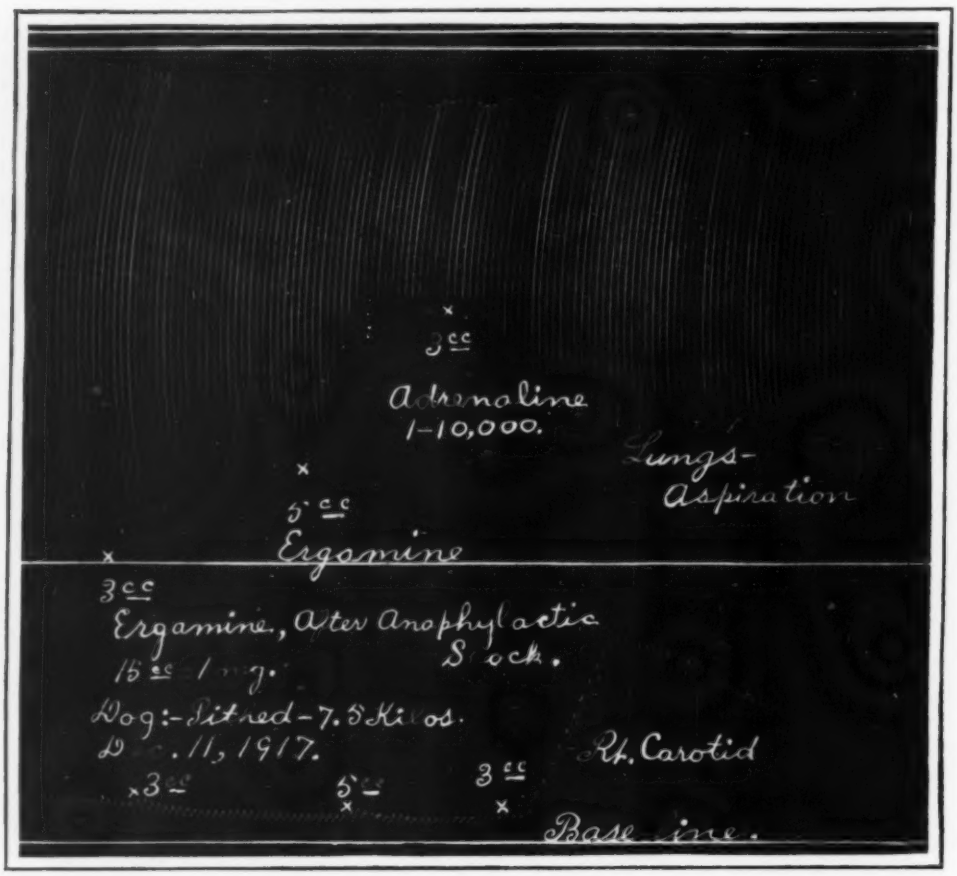

Fig. 4.

isolated liver of a sensitized dog when perfused with blood containing antigenic substance causes the blood to pass out of the organ with its coagulability either diminished or absent, while injection of antigen into one branch of the portal vein produced certain symptoms of shock and a profound congestion of that part of the liver supplied by the vein injected. Manwaring's results were obtained by means of side-tracking the portal blood by connecting the portal and external jugular veins and permitting the portal blood to flow directly to the heart, hirudin being used to prevent coagulation. Voegtlin and Bernheim made use of animals with an Eck fistula and combined this with ligation of the portal

vein near the hilus of the liver, the hepatic artery being temporarily clamped off. Both Manwaring, and Voegtlin and Bernheim in the animals which they so prepared, observed no symptoms of anaphylactic phenomena, and have accordingly considered the reactions of anaphylactic shock to be dependent on the liver, and not to occur without its activity.

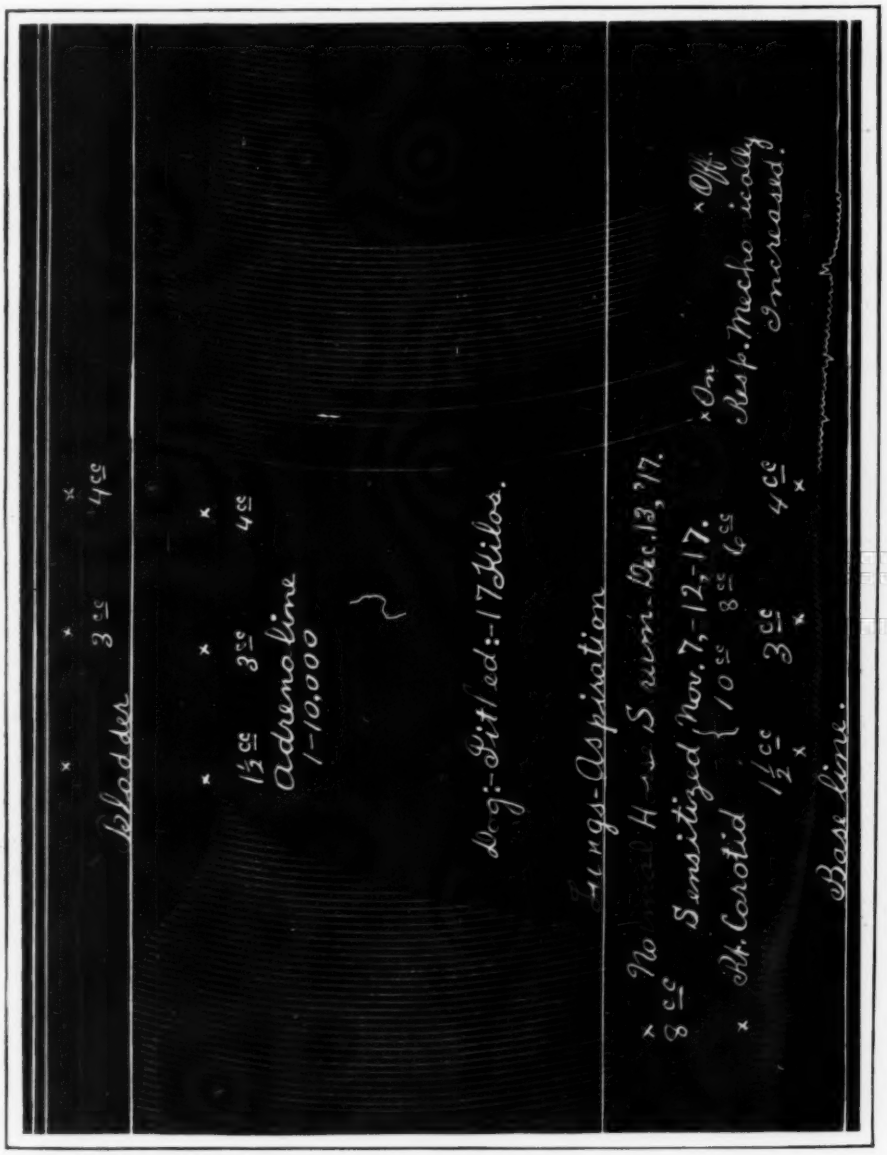
Bladder 3 cc 4 cc
1½ cc 3 cc 4 cc
Adrenaline
1-10,000
Dog:- Fitted:- 17 Kilos.
Lungs- Aspiration
Horse H - S. rum - Dec. 13, '17.
8 cc Sensitized Nov. 7, - 12, - 17.
R. Carotid 1½ cc 3 cc 4 cc
10 cc 8 cc 6 cc
Base line.
Disp. much readily increased.
Off.

Fig. 5.

In our experiments we were able to obtain the typical anaphylactic reaction without the liver and also without the aid of any structures below the diaphragm. This was accomplished by clamping above the diaphragm the vessels carrying

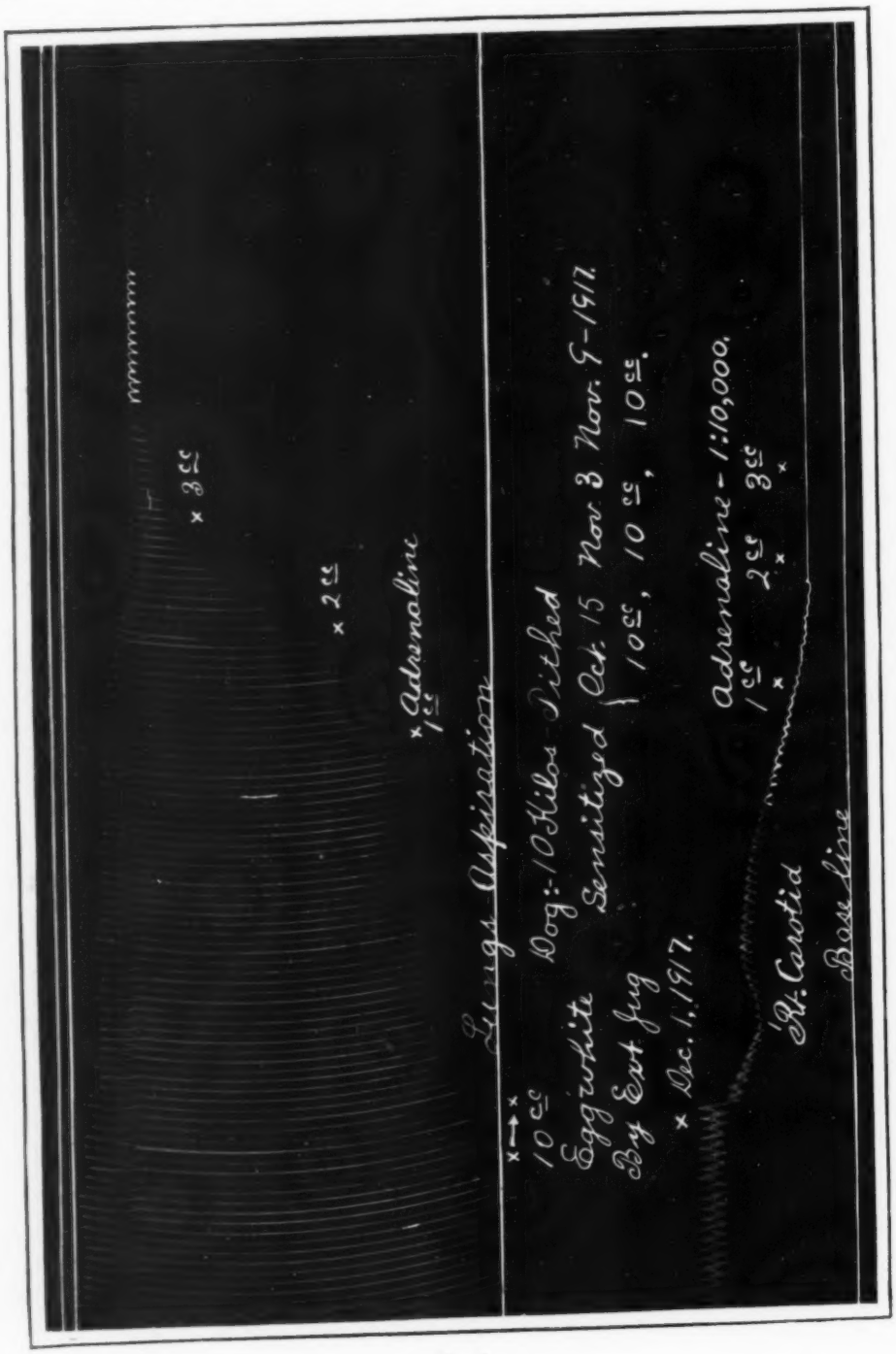

Fig. 6.

blood to and from the abdomen. The dogs were prepared as described above, and through the open chest was introduced a stout string threaded on two large needles. At a distance of about two centimeters laterally from each side of the

vertebral column and about five centimeters above the diaphragm the needles were pushed through intercostal spaces in the posterior chest wall to the exterior. They were then passed through holes in the dog board just beneath their point of exit from the chest. By drawing on the two ends of the string below the dog board and then clamping them, the aorta and azygos veins could be tightly

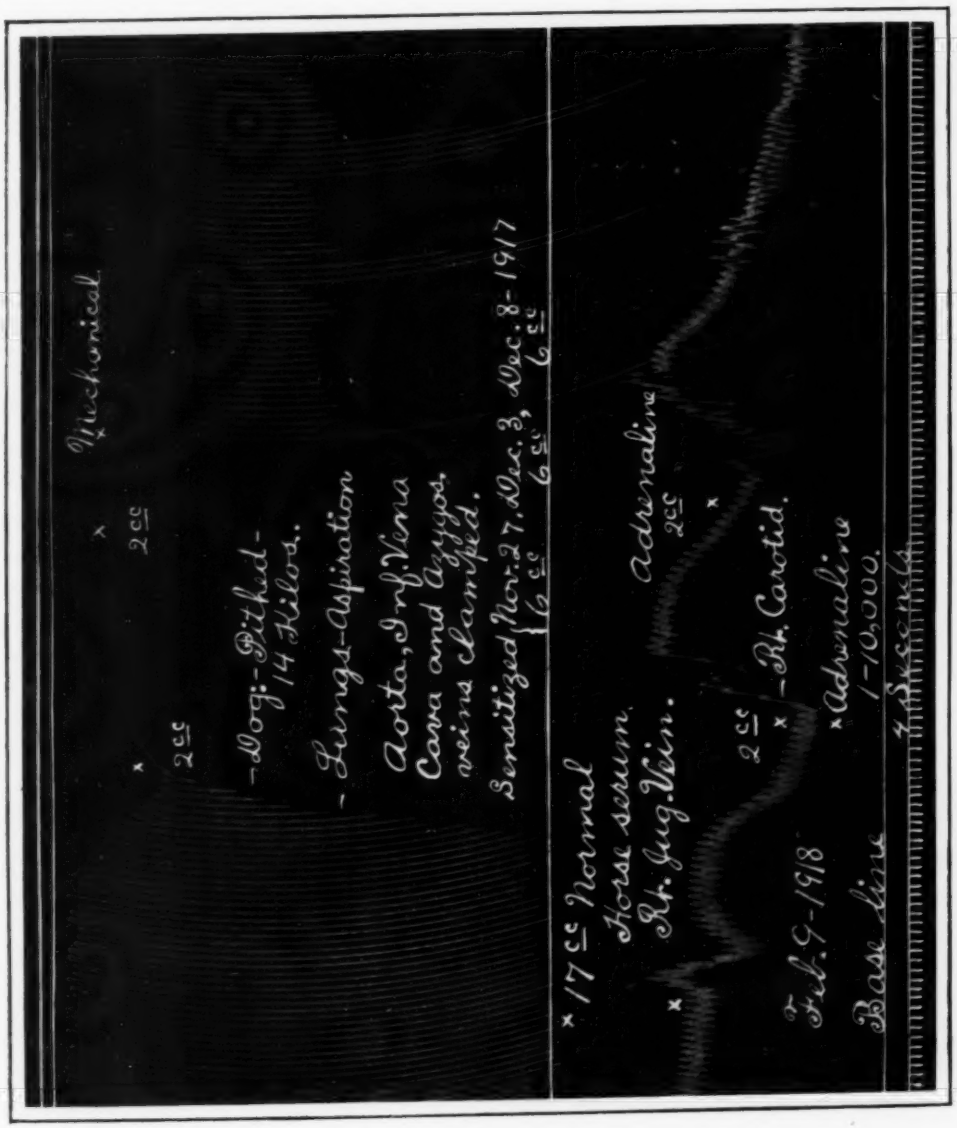

Fig. 7.

pressed against the vertebral column, and in this manner occluded. The inferior vena cava was ligated just above the diaphragm. The mammary arteries and veins were ligated when the chest was opened. Thus was circulatory communication with the abdomen completely stopped. This was verified by section of the femoral arteries. Then proceeding as in the experiments above described we in-

jected the antigen into the external jugular vein, and obtained a profound bronchoconstriction and a typical fall in blood pressure as usual (Fig. 7). Evidently the liver or intestines had nothing to do with these reactions which occurred exactly as in the intact animal.

In all of our experiments, in addition to obtaining the graphic record of the bronchial constriction we were able in looking through the window of the apparatus in the chest wall to observe the contraction and shrinkage of the lungs to about one-third their previous size, upon injection of the antigen. Apparently a very marked bronchoconstriction occurs in every case if the dog has been thoroughly sensitized. The criterion by which we have assumed the presence or absence of sensitization is the same as that used by preceding observers,[3] namely the fall in blood pressure.

We have as yet had no opportunity to study the split protein products of Vaughan,[14] which, as the splendid experimental work of Edmunds[4] has indicated, will at some future time, probably yield further interesting results along the lines indicated above.

CONCLUSIONS

1. From the experimental evidence at hand we may be certain that upon the injection of the antigen in highly sensitized spinal dogs, the asphyxia produced by the acute bronchial constriction may readily be the cause of death, and that the phenomenon occurs with the liver and other abdominal viscera wholly removed from the circulation.

2. The phenomena occur in those dogs sensitized to egg white equally as well as in those sensitized to normal horse serum.

3. The bronchial and blood pressure changes produced in acute anaphylactic shock bear a striking and almost complete resemblance to those produced by certain opium alkaloids, such as codeine, heroine, peronine, etc.

4. For producing relaxation of the bronchioles, and aiding in recovery in acute anaphylactic shock, adrenaline is the most dependable substance, but to produce the best effects the drug must be injected early, before the bronchoconstriction has had time to become too intense.

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STUDIES ON IMMUNITY WITH SPECIAL REFERENCE TO COMPLEMENT FIXATION*

By Alfred Blumberg, Salt Lake City, Utah.

THE great assistance rendered by the complement-fixation method in establishing a correct diagnosis has made this test one of extraordinary importance. Its general use in the detection of various diseases, and in proper differential diagnosis, has lead to its study by many investigators. Their reports have had a tendency to confirm the correctness of the Bordet-Gengou method, which was first applied by Bordet and Gengou in the investigation of the B. typhosus, the B. coli communis, the micrococcus intracellularis meningitidis, the B. pertussis, the B. anthracis and the B. tuberculosis. These tests were conducted to prove the correctness of the first findings, but the actual investigation of the possibilities of diagnosing pathologic conditions was conducted by Bordet, Gengou and others—Bordet-Gengou,[1] pertussis; Widal-LeSourd,[2] typhoid; Foix, mallein,[3] scarlatina; Schleissner, scarlatina.[3]

It is not important to cite a historical outline of further experimental work, in that the results are well known, not only in serologic laboratories, but also to the physician and veterinarian. Both professions appreciate the value of the test. One has but to look over the conditions—syphilis, gonorrhea, glanders, infectious abortion in cattle, pertussis, dourine, and tuberculosis—to understand the enthusiasm which the practical application of the modified complement-fixation method aroused. To these conditions we may add the test used for the identification of blood and meat-food in medicolegal cases. Although the manner of conducting the various tests is analogous, there is a difference in methods which enables the laboratory worker to draw his own conclusion as to the presence or absence of a certain disease. This difference lies exclusively in one of the reagents—*the antigen.*

In considering the antigen, it is not necessary to go into familiar theoretic details, but a few words about certain features interesting enough to be specially mentioned, are not out of place. Syphilis, gonorrhea, glanders, infectious abortion in cattle, pertussis, dourine, and tuberculosis can be diagnosed by the use of a different antigen for each disease. There are three specific groups of antigens:

Group A is composed of antigens which contain the specific organisms of a certain disease. These organisms are emulsified in saline, or the autolysate of the organisms is used.

Group B consists of an antigen which is essentially the liquid culture of a specific organism, and

Group C contains antigens which are the watery or alcoholic extracts of tissues.

In the diagnosis of gonorrhea, pertussis, glanders, infectious abortion in

*The author wishes to express his appreciation to Marcus Ward Lyon, Jr., Ph.D., M.D., Professor of Bacteriology and Pathology, George Washington University, Washington, D. C., for suggestions and especially for the arrangement of the zoological nomenclature.

cattle, and dourine, antigens belonging to the first group are used. It is very interesting to note that these conditions are accompanied by a polymorphonuclear leucocytosis, or at least by a local pus condition. Dourine, in which there may be a lymphocytosis present, is, perhaps, an exception. Since the antigens already contain the etiologic factor of the respective diseases, they are specific antigens. Certain authors are inclined to consider the possibility of conducting successful complement-fixation by emulsifying the tubercle bacilli, and using the emulsion as an antigen in the detection of tuberculosis. Claims as to the results obtained are surprisingly optimistic, but can not be confirmed in certain instances. *A lymphocytosis is usually present in tuberculosis.*

There is but one antigen belonging to Group B in use, namely, the liquid egg medium tuberculosis culture of Besredka.[4] This antigen is considered to be specific, and will be referred to later in this study.

There is only one antigen of Group C deserving special mention—the antigen for syphilis, which was at first considered a true one, as it was prepared from an organ containing the etiologic factor of the disease. It was soon found, however, that it was the lipoid material, and not the treponema pallidum, which was responsible for the reaction. *Syphilis is accompanied by a lymphocytosis.*

Although complement fixations are classified in three groups, there is a fourth group—that of complement fixation without the presence of any antigen.

In considering a special antigen, the one for the complement fixation of tuberculosis has been selected. Some investigators make use of bacterial emulsions for antigenic purposes only, the foremost advocates in this direction being Zinsser and Miller,[5] who conducted their tests with an emulsion containing tubercle bacilli suspended in hypotonic salt solution. Eichhorn and Blumberg[6] could not substantiate the findings of Zinsser and Miller. The test conducted by Eichhorn and Blumberg dealt with sera derived from cattle; it was, therefore, necessary to conduct tests with sera of human origin. Altogether sixty sera were used for this purpose, the findings of which are found in Table I. The apparent objection was that the antigenic and the anticomplementary titers were too close. One other bacterial emulsion antigen has been in use in connection with comparative work conducted with the antigen of Zinsser and Miller. This bacterial emulsion (known as Friedmann's vaccine) contained the ichtyc type of the tubercle bacilli, but the results showed too many positives (See Table I). In establishing the diagnostic possibilities of a disease in which there is a lymphocytosis, these antigens were used to represent antigens of the first group, which were controlled by antigens of the second group. The latter antigen was that recommended by Besredka[7]—the liquid egg medium antigen, the preparation was the one modified by Eichhorn and Blumberg.[6]

It was found that the liquid culture when freed from the tubercle bacilli by means of Berkefeld filtration, did not present satisfactory antigenic properties. It is absolutely necessary to heat the culture before filtering it, but the heat applied is not enough to destroy any component properties of the antigen. It seems that the heat aids not only in the extraction of the organisms, but in the fine distribution of the fat. It must be remembered that since there is lipoid material present in the antigen, it may, in a luetic condition, have a tendency to give positive reactions. Bronfenbrenner[8] was perhaps one of the first in this

country to observe and study this phase of Besredka's antigen. There is, however, a possibility of overcoming a nonspecific reaction. The first step is to conduct a Wassermann test in every case where a positive reaction has been found; if the Wassermann test is also positive, the test for tuberculosis is repeated as before, except that three units of complement are used. With a few exceptions a modification of this type gives the desired results; negative reactions are obtained with nontuberculous sera derived from syphilitic individuals, and if positives are obtained in doubtful tuberculous cases, the reaction is considered positive for tuberculosis. This antigen has the advantage of being stable when ordinary precautions are taken. The following table gives the results obtained:

TABLE I

SUMMARY OF THE TESTS ON HUMAN SERA CONDUCTED WITH VARIOUS ANTIGENS FOR COMPARATIVE PURPOSE

CONDITION	ANTIGENS	XXXX	XXX	XX	X	–	TOTAL
1. Apparently healthy	Miller and Zinsser	3	1	2	4	16	
	Friedmann	3	6	–	7	10	
	Liquid egg medium	–	2	–	2	22	26
2. Clinical symptoms suspicious	Miller and Zinsser	2	1	1	1	4	
	Friedmann	8	1	–	–	–	
	Liquid egg medium	4	1	1	–	3	9
3. Clinical symptoms moderate	Miller and Zinsser	5	3	1	–	4	
	Friedmann	11	1	1	–	–	
	Liquid egg medium	5	2	5	–	1	13
4. Clinical symptoms progressive	Miller and Zinsser	3	–	2	–	2	
	Friedmann	7	1	–	–	–	
	Liquid egg medium	4	–	1	–	2	7
5. Clinical symptoms severe	Miller and Zinsser	–	–	–	1	4	
	Friedmann	5	–	–	–	–	
	Liquid egg medium	–	1	–	–	4	5
							60

Although Craig[9] reported impressive results, experiments were not conducted with his antigen, because in private communication,[10] he stated that the antigen prepared by him was so unstable that it was impossible to preserve it for more than a few hours, even though the utmost precautions were taken. To prepare a reagent identical to the one described by Craig was considered too difficult, and the experimental work in this direction had to be given up. Craig has recently adopted a modification of his antigen and published the results of his experiments on the subject.[11]

Other experiments were conducted in connection with those on complement fixation of tuberculosis, to determine the possibilities of diagnostic aids. Szaboky[12] stated that there were moments in the phase of the disease when agglutinations were demonstrable. Forty sera were tested with bacterial emulsions of the Zinsser-Miller and Friedmann types without noticeable reactions. In some instances the Friedmann vaccine presented a slight clumping, but of insufficient value to make it practical for diagnostic purposes.

The demonstration of precipitins was conducted with three different re-

agents: (1) The supernatant fluid of the Zinsser-Miller antigen, obtained by centrifugalizing the antigen; (2) the supernatant fluid of Friedmann's vaccine, obtained by centrifugalizing the vaccine; and (3) pseudoglobulins prepared from the serum of tuberculous cattle. The first two reagents were negative and consequently valueless, the practical value of the third is very limited. Forty sera were tested for precipitins; the Friedmann organism extract showed a fine ring not always of the same intensity.

The *conglutination test* was applied experimentally by Strengs[13] in the diagnosis for syphilis, and was first conducted in this country by Wehrbein[14] for the diagnosis of dourine. This method was used by the writer in detecting its practical value as related to tuberculosis. Altogether twelve sera were used for standardizing purposes; the practical value of this test in the diagnosis of tuberculosis was absolutely unsatisfactory. The accompanying tables show the methods of standardization:

TABLE II

TITRATION OF ANTIGEN

NO. OF TUBE	SALT SOLUTION	COMPLEMENT	SERUM	ANTIGEN
1			T. B. serum 0.1	0.025
2			0.1	0.05
3	Sufficient	As	0.1	0.1
4			0.1	0.15
5	quantity	determined	0.1	0.2
6			0.1	0.25
7	to	by	0.1	0.3
8			0.1	0.35
9	make 1 c.c.	titration	0.1	0.4
10			0.1	—
11			Normal S. 0.1	0.10
12			0.1	0.20
13			0.1	0.30
14			0.1	0.40
15			0.1	—

After one hour at 37° C. add ox serum and emulsion of sheep blood.
The result is read after three hours at 37° C.

TABLE III

TITRATION OF OX SERUM

NO. OF TUBE	SALT SOLUTION	FRESH HORSE SERUM COMPLEMENT	INACTIVATED OX SERUM TITRATED 1:10	SHEEP EMULSION 5%
1	0.75	0.1	0.15	0.1
2	0.8	0.1	0.1	0.1
3	0.15	0.1	0.075 = 0.75	0.1
4	0.4	0.1	0.05 = 0.5	0.1
5	0.65	0.1	0.025 = 0.25	0.1
6	0.8	0.1	0.01 = 0.1	0.1
7	0.9	—	—	0.1
8	0.9	0.1	—	0.1
9	1.0	—		0.1

Three hours at 37° C.
Twice the smallest amount giving complete conglutination is used as titer.

In conducting complement fixations for diagnostic purposes with antigens of the third group (essentially of tissue extract origin), not only syphilis, but

tuberculosis and rabies were diseases towards which investigators looked with interest. Hammer[15] believed in the possibilities of an antigen derived from tuberculous organs in diagnosing tuberculosis, while Zell,[16] using the extracts of submaxillary and parotid glands of rabid dogs, believed that he could successfully demonstrate rabies by means of complement fixation. That Zell's assertion was incorrect has finally been settled; and since the studies of Schöning[17] concerning Zell's findings did not in any way substantiate his hopes, syphilis is the only disease in which tissue extracts can be applied for diagnostic purposes. There are many variations in the preparation of such extracts, those most widely used being the ones prepared from the guinea pig, or beef or human hearts. Some are common alcoholic extracts, some alcoholic extracts reinforced with cholesterol, and others are the acetone insoluble fractions of the alcoholic extracts.

There is much dispute as to the comparative values of the several antigens in conducting the test. Most of the disputes, however, turn around the nonspecificity and inconsistency of the reactions in certain instances; true enough, the antigen is not specific insofar as no specific organisms are present, yet in

TABLE IV

TITRATION OF COMPLEMENT

NO. OF TUBE	SALT SOLUTION	FRESH HORSE SERUM DILUTED 1:10	INACTIVATED OX SERUM	SHEEP BLOOD EMULSION 5%
1	0.8	0.1	two units	0.1
2	—	0.09 = 0.9	" "	0.1
3	0.1	0.08 = 0.8	" "	0.1
4	0.2	0.07 = 0.7	" "	0.1
5	0.3	0.06 = 0.6	" "	0.1
6	0.4	0.05 = 0.5	" "	0.1
7	0.5	0.04 = 0.4	" "	0.1
8	0.6	0.03 = 0.3	" "	0.1
9	0.7	0.02 = 0.2	" "	0.1
10	0.8	0.01 = 0.1	" "	0.1
11	0.9	0.1	—	0.1
12	0.9	—	two units	0.1
13	0.85	0.1	one unit	0.1
14	1.0	—	—	0.1

The result is read after three hours at 37° C.
The smallest amount of horse serum giving complete conglutination is the titer.

the hands of accurate workers, the test is of inestimable value. It is also true that there is a certain inconsistency in the test which expresses itself, not so much in excessively positive reactions, as in the reverse. Kaplan,[18] who believes in proving a negative reaction, states that, when conducting his tests, he added four units of amboceptor to the tube. There is no objection to this; as a matter of fact, the very first authors on the subject of complement fixation applied similar methods,[19] probably under the same impulse. Although such negative provocative tendencies may suggest the general occurrence of excessive false positive Wassermann reactions, this is not the case. Conditions often arise where the Wassermann test presents a negative reaction, even when the patient is known to have had syphilis and probably still has it. A more vigorous antigen was looked for as a remedy, and it was finally agreed that a cholesterol

reinforced antigen would meet the requirements. Today no test is performed without a cholesterolized antigen control. It must be added, however, that contradiction very often occurs concerning the reactions which result from the use of cholesterolized and noncholesterolized antigens. On many occasions positive tests are present with the cholesterolized antigen, where the noncholesterolized antigen presents negative results. Such a condition would cause no alarm if the cholesterolized antigen would present a specific and early reaction in suspicious cases, unfortunately no considerable number of early findings can

TABLE V

MAMMALS

Ferruginous Tree-shrew, *Tupaia glis ferruginea*, Singapore	0.15...0.20
European Hedgehog, *Erinaceus Europeus*	0.15...0.10
Star-nosed Mole, *Condylura cristata*, Maryland	0.05...0.05
Luzon Hairy-tailed Shrew, *Crocidura luzonensis*, Manila	0.05...0.15
Murino Hairy-tailed Shrew, *Crocidura murina*, Baltistan	0.05...0.20
Fish-eating Bat, *Noctilio leporinus*, South America	0.05...0.40
Papuan Emballonurine Bat, *Embellonura nigrescens*, Papua	0.10...0.20
Blood-sucking Bat, *Desmodus rotundus*, Ecuador	0.05...0.50
Hoary Bat, *Nicterus cinereus*, New York	0.15...0.6
Florida Free-tailed Bat, *Tedarida cynocephalus*, Florida	0.10...0.40
Norway Rat, *Rattus norvegicus*, Manila	0.025..0.15
Pocket Gopher, *Geomys bursarius*, Minnesota	0.05...0.15

TABLE VI

BIRDS

Tufted Puffin, *Lunda cirrbata*	0.12...0.80
Kittiwake Gull, *Rissa*	0.15...0.55
Pelican, *Pelecanus*	0.05...0.40
Oyster-catcher, *Hæmatopus*	0.20...0.80
Jacana, *Jacana*	0.15...0.20
Goshawk, *Astur Palumbarius*	0.10...0.20
Osprey, *Pandion*	0.025..0.050
Yellow-headed Blackbird, *Xanthocephalus*	0.15...0.40
Bobolink, *Dolichonyx oryzivorus*	0.25...1.00
Waxwing, *Ampelis*	0.15...0.20

TABLE VII

REPTILES

Mons Island Ground Iguana, *Cyclura stejnegeri*, Mona Island, Porto Rico	0.10...0.25
California Fence Lizard, *Sceleporus biseriatus*, California	0.05...0.40
Boa Constrictor, *Boa Constrictor*, South America	0.20...0.80
Hog-nosed Snake, *Lystrophis d'orbignyi*, Brazil	0.20...0.20
Asiatic Snake, *Dinodon rufozonatum*, Eastern Asia	0.15...0.30
Chicken Snake, *Elaphe confinis*, Wisconsin	0.15...0.20
Band-tailed Chicken Snake, *Elaphe taeniurus*, Lorea	0.10...0.20
Mud Turtle, *Cinosternon flavescens*, Texas	0.025..0.50

be obtained with regularity, yet I have more than once obtained a positive test with a noncholesterolized antigen, and a negative one with a cholesterolized antigen using the serum of patients presenting primary lesions.

The study of the possibilities of testing tissue extracts obtained from sources

other than beef or guinea pig heart, or human liver or heart was of great interest. Thirty-seven varieties were tested for antigenic and anticomplementary doses; these specimens included extracts from 13 mammals, 10 birds, 8 reptiles, and 7 fishes. Tables V, VI, VII, and VIII will show the animals from which the extracts were obtained, and the results.

TABLE VIII

FISHES

California Skate, *Raja bonoculata*, British Columbia	0.15	0.40
Cow Shark, *Notorhynchus maculatus*, San Francisco	0.15	0.20
Moray Eel, *Mursena nubila*, Asia	0.05	0.10
Cut-throat Trout, *Salmo mykiss*, Montana	0.20	0.80
Whitefish, *Leucichthys harengus*, Lake Superior	0.05	0.35
Common Carp, *Cyprinus carpio*, Texas	0.05	0.10
Great Club, *Leucisus lineatus*, Utah	0.05	0.15

That the values of the above findings are somewhat relative in character can not be denied, since there was no uniformity in the preparation of the extracts. Then, too, these extracts were not prepared for a special purpose; they were nothing more than the alcohol in which museum specimens of the above animals were preserved. One point, however, is of great importance; while there were a few extracts which had no antigenic value, there were some of decidedly valuable character. Whether or not the proportion tends to throw light upon the identities of cell characteristics in various animals can not, at present, be stated.

In connection with the complement fixation results, it is customary to speak of four, three, two, and one plus positives. Various attempts to apply methods for the determination of the strength of positive reactions were made, the most popular being that of centrifugalizing the blood corpuscles which remained unhemolyzed, and, by means of a scale system engraved on the centrifuge tube, determining the amount of corpuscles present, and the strength of the reaction. This method, while easily applied, is by no means faultless, and it seems that decreasing amounts of red blood corpuscles added to a set of tubes (containing the questionable serum, the antigen, the complement and the hemolysin) may serve to give more accurate results where they are desired. The following table demonstrates the method advised for the determination of the positive strength of a given serum.

If a serum does not hemolyze the amount present in the tenth tube, it is considered 100 per cent positive. The serum which hemolyzes Tube 10, but does not affect Tube 9, is considered 90 per cent positive; thus a serum hemolyzing to the fourth tube, No. 4 not being hemolyzed, however, is considered 40 per cent positive; the serum of the tube containing 0.9 c.c. blood emulsion, if incompletely hemolyzed, while the previous tube has been completely hemolyzed, is considered 10 per cent positive.

This titration method is of significance only in ascertaining the condition of a patient who has undergone treatment, and whose reaction was strongly positive before treatment.

TABLE IX

TITRATION OF SERUM FOR COMPLEMENT BINDING POWER

NO. OF TUBE	SERUM	ANTIGEN	COMPLEMENT 5% SOLUTION	SALINE	INCUBATION TIME	HEMOLYSIN 2 UNITS IN 1 C.C.	SHEEP BLOOD EMULSION 5%	INCUBATION TIME
1	0.2	As determined by titration	1 c.c. to each tube	Sufficient quantity to make 3 c.c.	1 hour at 37° C.	Added to each tube after incubation time	1.0	1 hour at 37° C.
2	0.2						0.9	
3	0.2						0.8	
4	0.2						0.7	
5	0.2						0.6	
6	0.2						0.5	
7	0.2						0.4	
8	0.2						0.3	
9	0.2						0.2	
10	0.2						0.1	

THE COMPLEMENT FIXATION WITHOUT ANY ANTIGEN

Deluca[20] stated that in setting up a hemolytic system, when the urine of a pregnant was added, he succeeded in obtaining hemolysis, but in the presence of normal urine, the same system would not show hemolysis. The diagnosis of pregnancy by laboratory methods was very welcome after Abderhalden[21] had published his method. There was, of course, a great difference in the bases of his work, and that conducted in the present study. Abderhalden based his work on the specific enzymes present in the blood under certain conditions, and the demonstration of the enzymes was the essential basis of his method. Unfortunately many things rendered Abderhalden's test unpopular, and anything of more certain characteristics, and simple technic would be received with great appreciation by the clinician. Two hundred fifty-nine samples of urine were tested by the method here described, the results being so encouraging that the continuation of the study of the test seems to be of high importance.

The technic of the method is as follows: to 10 test tubes containing increasing amounts of urine, one-half c.c. of 20 per cent complement, 0.1 c.c. of 10 per cent sheep-blood emulsion and two units of hemolysin are added. The amount of urine contained in the first tube presenting complete hemolysis is the titer. The values found by the titration of urines are nearly absolute, in that once standardized to a certain hemolytic system, the titer of urines is constant.

To conduct the test, three tubes are set up for each specimen to be tested. The tubes are size No. 1, as used in the Noguchi test. The first tube contains the complete hemolytic system and 0.15 c.c. of the urine; the second contains the complete hemolytic system, and 0.25 c.c. of urine, while the third contains 0.25 c.c. of the urine, and the hemolytic system without complement. The last tube is the control tube. It is not necessary to have a second control tube which contains complement but no amboceptor; the absence of amboceptor does not, in many instances, prevent effective hemolysis. To each tube, 1 c.c. of physiologic saline is added, after which the tubes are well shaken and the test put in the incubator at 37° C. In case no hemolysis results within one hour, the test is considered negative for pregnancy, but if hemolysis occurs, the test is positive, the latter reaction usually setting in within the first 25 to 30 minutes. In some

instances instantaneous hemolysis occurs. If there is hemolysis present in the third tube, it is due to some cause other than pregnancy. There is no difficulty in reading the test, because unless the hemolysis is complete, there is no hemolysis present. The absence of hemolysis denotes absence of pregnancy; this point must be emphasized, in that so far I have experienced no test in which the urine of a pregnant did not present hemolysis.

Although the presence of hemolysis usually means pregnancy, there are certain exceptions (see tables below) which are, as a rule, of a type such that differential diagnosis is not unpractical.

TITRATION OF NORMAL URINE FOR INHIBITING PROPERTIES: AND TITRATION OF URINE OF A PREGNANT FOR HEMOLYTIC PROPERTIES

The accompanying tables demonstrate the method of titration for the inhibiting and hemolytic properties:

TABLE X

TITRATION OF PREGNANCY URINE

NO. TUBE	URINE	COMPLEMENT	HEMOLYSIN	10% R. B. C.	SALINE	RESULT
1	0.05	One-half c.c.	0.1 c.c. rep-	0.1 c.c.	1 c.c.	Com. hemolysis
2	0.10	representing	resenting	for each	saline	Com. "
3	0.15	2 units to	2 units to	tube	to each	Com. "
4	0.20	each tube	each tube		tube	Com. "
5	0.25					Com. "
6	0.30					Incomplete
7	0.35					No hemolysis

TABLE XI

TITRATION OF NORMAL URINE

NO. TUBE	URINE	COMPLEMENT	HEMOLYSIN	10% R. B. C.	SALINE	RESULT
1	0.05	One-half c.c.	0.1 c.c. rep-	0.1 c.c.	1 c.c.	No hemolysis
2	0.10	representing	resenting	for each	saline	" "
3	0.15	2 units to	2 units to	tube	to each	" "
4	0.20	each tube	each tube		tube	" "
5	0.25					" "
6	0.30					" "
7	0.35					" "

A few cases may illustrate the possibilities of applying the complement-fixation test for diagnostic purposes.

Case No. 1.—Mrs. H., last menstruation, Dec. 25, 1916; test performed Jan. 30, 1917, reaction positive. Symptoms of pregnancy set in two weeks later.

Case No. 2.—Mrs. W. C., age thirty-eight, last period Oct. 28, suspected change in life; called on physician Jan. 17, 1917. Reaction positive. Found to be gravid on examination.

Case No. 3.—Patient thirty-eight years of age; suspected change of life; no children before. Last menstruation four weeks previous; called on physician, reaction positive. Found to be pregnant three weeks later.

Case No. 4.—Mrs. K., two months since last menstruation, reaction positive. On operation found to be gravid about two months.

Case No. 5.—Patient twenty-eight years old, appendicitis, reaction positive, on operation found to be pregnant two months.

Case No. 6.—Girl, diagnosed to be pregnant, denied condition or possibilities, changed physician. Reaction negative. Examination negative. Diagnosed later, tuberculosis of mesentery.

These cases were selected for demonstration, a summary of the findings being given in Table XII.

TABLE XII

CONDITION	POSITIVE REACTION	NEGATIVE REACTION	TOTAL
Normal men	–	37	37
Girl child	–	1	1
Normal women of proper age	–	12	12
Women above age	–	2	2
Pregnancy present			
Condition first month	14	–	14
" second	8	–	8
" third	6	–	6
" fourth	2	–	2
" fifth	7	–	7
" sixth	7	–	7
" seventh	7	–	7
" eighth	5	–	5
" ninth	10	–	10
Pregnancy, no history of time	6	–	6
After fourth day of delivery	–	4	4
After ectopic pregnancy over 8 days	–	1	1
Pregnancy and albuminuria	1	–	1
Pregnancy and other pathologic condition	2	–	2
Casts in urine no albumin present	1	–	1
Albumin in urine no casts present	1	–	1
Albumin and casts in urine	1	–	1
Pus in urine	8	–	8
Sugar in urine	–	4	4
Hematuria	–	2	2
Appendicitis	1	27	28
Tuboovarian abscess	–	2	2
Pyosalpinx	–	2	2
Pyelonephritis	1	–	1
Vaginal abscess	–	1	1
Epidemic meningitis	–	1	1
La grippe	–	1	1
Measles	2	–	2
Pneumonia	–	1	1
Syphilis secondary	–	3	3
Tabes	–	2	2
Tuberculosis	1	5	6
Variola	1	–	1
Cerebral hemorrhage	–	1	1
Before chololithotomy	–	2	2
Before gastroenterostomy	–	1	1
Before hysterectomy	–	1	1
Gastric ulcer	1	2	3
Stenosis of the pylorus	1	–	1
Cardiac insufficiency	2	1	3
Mucus gastritis	–	1	1
Phlebitis	–	1	1
Varicose veins	–	1	1
Hemorrhoids	–	1	1
Colloid goiter	–	2	2
Myxedema	1	–	1

TABLE XII.—CONT'D

CONDITION	POSITIVE REACTION	NEGATIVE REACTION	TOTAL
Adenoma	–	1	1
Myoma	–	1	1
Carcinoma	–	1	1
Secondary anemia	–	1	1
Injuries	–	5	5
Chemicals taken:			
KI	–	2	2
Salvarsan	–	4	4
Men under ether	1	7	8
Cases without history	–	6	6
Old urines	1	1	2
Differential diagnosis between tumor and pregnancy and diagnosis found correct	–	2	2
Suspected pregnancy, diagnosis found to be correct later	22	8	30
After operation	1	5	6
Alcoholics	1	5	6
After removal of kidney of man	–	1	1
Edema of face	–	1	1

The question naturally arises, What is the mechanism of the test? Is it a true complement fixation, or is the change strictly of a chemical nature? As the investigations in this direction are not finished, no final answer can be given. The first step in proving that the change was not due to physical influences, was to determine the specific gravities of the various specimens. As a result it can be safely stated that specific gravity has scarcely any bearing on the test. With a specific gravity as high as 1.035 urines have given complete hemolysis, and urines with a specific gravity of 1.007 have failed to give results. Since the addition of excessive amboceptor would not cause changes in the reaction if no hemolysis were previously present, it seems probable that the test is that of a true complement fixation, but, on the other hand, if pregnancy is present, hemolysis will set in—perhaps with a slight delay—even if no amboceptor is added. The atypical reaction is demonstrable in urines of patients who suffer from some type of nephritis. The fact that conditions like smallpox, scarlatina or measles will present a condition which results in the hemolytic effect of the urine, while the urine itself is free of casts or albumen, is by no means a contradiction. These diseases may cause an initial lesion—perhaps temporary in type—which does not cause symptoms severe enough to produce albumin or casts, but is detectable by means of a seroreaction. As a matter of fact, in no case where there are casts present, will there be a lack of hemolysis, and in a few instances only will there be inhibitions presented where pus or albumin is found; in all these instances pus and albumin are due to a diseased kidney.

The above statistics tend to show that syncytial toxins present in the circulation of the gravid woman will affect the kidneys, the intensity of the nephritis depending upon the intensity of the toxin. Urines from such patients do not inhibit hemolysis, whether or not these urines are high in concentration.

The fact that pathologic conditions of other types do not present false tests, and that, unless the urine is old, no fixation of complement will set in where pregnancy is present, renders this test worthy of serious consideration.

CONCLUSIONS

1. We have shown that true antigens (e.g., antigens which contain the etiologic factor of the disease, emulsified or autolyzed) will work only where there is a polymorphonuclear leucocytosis present.

2. While tuberculosis is a disease presenting a lymphocytosis, it will fix the complement with a specific antigen, which, however, is not a bacterial emulsion, but a heated egg medium culture containing small amounts of lipoid.

3. Tissue extracts of mammals, birds, reptiles and fishes may serve as useful antigens for the diagnosis of syphilis.

4. If normal urine is added, the complement of the hemolytic system is affected without the presence of a specific antigen.

5. The presence of hemolysis in the test of the fourth group indicates either some type of affection of the kidney (even when no albumen or casts are demonstrable) or pregnancy, the two conditions frequently being separable by the clinical history of the patient.

6. The absence of hemolysis in a hemolytic system to which urine is added speaks against the condition of pregnancy.

7. In complement fixation without an antigen, reaction affecting the third tube (which serves as a control tube and should not hemolyze) speaks for nephritis, rather than pregnancy.

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MENINGITIS AT CAMP GREENE

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CEREBROSPINAL meningitis of the meningococcic type has been a matter of great concern in the training camps of the United States during the past winter. It is, therefore, useful to place on record such data regarding this disease as may be available in order that in future times more may be known of the epidemiologic occurrence of it, and that if possible the laws governing its spread may be worked out.

In Camp Greene the disease was at no time truly epidemic though in one organization at least it acquired something of the appearance of epidemicity. This will be evident from a glance at the table which shows that in a period of something over two months only thirty cases occurred in a camp population varying between 30,000 and 40,000. Such an incidence in such a population can not be called epidemic. To be strictly accurate there were but 29 cases, for one of the thirty (No. 21) was of the pneumococcic type as proved by bacteriologic examination. Under the conditions of camp life such as prevailed during the past winter, with exceedingly cold weather, with sleet and rain alternating, with the men practically living in the mud, wet from one day's end to another, huddling together for warmth,—in other words with all the best conditions for the transmission of infection,—such an incidence can not be called epidemic. As a matter of fact it is a surprising fact that the sick rate was as low as it has been, and the amount of diseases that occurred from all causes speaks well for the care exercised over the men.

Cases of meningitis began to appear in December, 1917, and after the appearance of the first case of the disease continued to appear sporadically during January and February as shown in the table.

Certain facts relating to some of these cases are interesting and are to be borne in mind in deciding to what extent the camp conditions were responsible for the persistence of the disease. For instance,—

Case No. 28 had not been in camp for at least a month before he was relieved from duty with the Provost Guard in Charlotte, N. C., and reported back to his organization. Two days after returning to camp he was taken ill with meningitis.

Case No. 24 had, like the preceding one, been on duty with the Provost Guard in Charlotte N. C. Immediately upon his return to duty with his organization at Camp Greene he was placed in quarantine with a group of measles contacts and sent to the Detention Camp. After eight days in quarantine he developed meningitis. None of his tent or squad mates had the disease.

Case No. 14 was a recent recruit who had been separated from his battery with a group of other recruits with whom he was being trained. He had been assigned to his regiment about eight days when he developed meningitis. None of the other members of his group developed the disease.

CASE NO.	DATE REPORTED	CO. OR BATT.	ORGANIZATION NO.	REMARKS
1	Dec. 18, 1917.	B	9	
2	Dec. 21, 1917.	I	8	
3	Jan. 3. 1918.	G	8	
4	Jan. 3, 1918.	L	19	
5	Jan. 4, 1918.	E	12	
6	Jan. 9, 1918.	L	8	
7	Jan. 9, 1918.	K	12	
8	Jan. 9, 1918.	M. G.	19	
9	Jan. 9, 1918.	L	19	
10	Jan. 11, 1918.	H	1	
11	Jan. 11, 1918.	A	9	
12	Jan. 12, 1918.	D	10	
13	Jan. 12. 1918.	F	14	
14	Jan. 15, 1918.	E	15	Recruit.
15	Jan. 16, 1918.	33 Amb. Co.	26	
16	Jan. 17, 1918.	A	16	
17	Jan. 17, 1918.	A	15	Recruit.
18	Jan. 18, 1918.	E	3	
19	Jan. 20, 1918.	D	1	Developed in Detention Camp.
20	Jan. 20, 1918.	K	5	
21	Jan. 25, 1918.	C	9	Pneumococcus case.
22	Jan. 29. 1918.	Hdqs. Co.	19	
23	Feb. 1, 1918.	M. G.	6	
24	Feb. 4, 1918.	B	15	Charlotte case.
25	Feb. 8, 1918.	K	10	
26	Feb. 8, 1918.	A	7	
27	Feb. 10, 1918.	Hdqs. Co.	12	
28	Feb. 11, 1918.	F	14	Charlotte case.
29	Feb. 12, 1918.	D	6	
30	Feb. 13, 1918.	C	15	

Case 17 was also a recruit who immediately upon his arrival at camp came in contact with a case of measles and was promptly sent to the Detention Camp where he later developed meningitis.

In none of these cases, certainly, did the regimental conditions have anything to do with the appearance of the disease, and, also, since each man was kept isolated with a different group of associates in none of whom meningitis appeared, the cases themselves were not factors in the further occurrence of the disease. They were in other words sporadic cases which did not become centers about which other cases developed. No plausible explanation of the original infections in the individual cases can be offered other than the one that in each case the causative organisms were present and merely waiting for the proper decrease of resistance of the individual before invading the tissues. It is a surprising fact that under the necessary conditions of living there were not many more cases. For days at a time the weather and ground conditions were such that the men could leave their tents only to perform the absolutely necessary duties. Drill was impossible and nothing remained for them to do but lie around in their tents trying to keep warm and waiting for a propitious day when their bedding and clothes could be aired. The conditions were the same for officers and men and the disease did not confine itself to the men.

It will be noted that an organization in which three of the cases occurred lay adjacent to another organization in which a number of other cases appeared.

It will also be noted that between these two organizations are two (17 and 18) in which there were no cases. In other words there seemed to be no evidence of spread from one regiment to a neighboring one.

The features connected with meningitis in organization No. 19 are unique for this camp. In this regiment the incidence suggested epidemicity, and also in it one company had more than one case of meningitis,—the only instance of this in the camp. In no instance, however, was there more than one case in a squad. In this regiment three cases were from Company L, and one each in the Machine Gun Company and Headquarters Company. One of the cases which developed in Company L had been detailed to the regimental infirmary where he had come in direct contact with the man from the Machine Gun Company who later developed the disease. This, so far as can be determined, is the only instance in Camp Greene in which immediate contact could be strongly suspected of being a factor in spreading the disease.

The only available statistics at hand indicate that there have been between 5 and 7 per cent of carriers in Camp Greene. These figures came from the

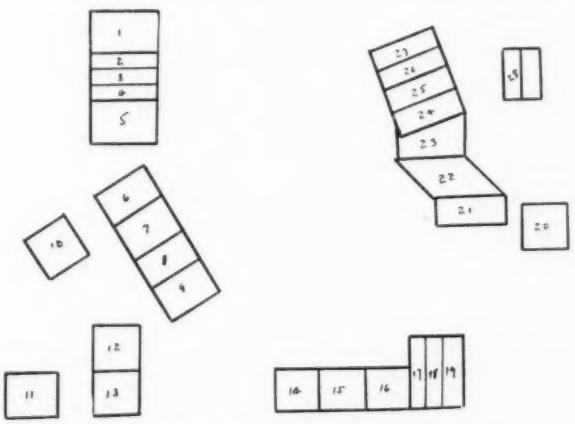

Chart to show the location of the different organizations in Camp Greene.

Detention Camp and are therefore too high, in all probability, for in this camp all meningitis carriers were isolated pending bacteriologic examination. It would appear therefore that the percentage of carriers in Camp Greene was smaller than in other camps. However that may be, it represents after all merely a guess because the only organizations which were cultured were those in which meningitis appeared and probably represents not more than half the personnel of the whole camp.

More interesting is the fact that in the only organization which made use of systematic nasal sprays since the first of the year not a single case developed, and also that in those organizations in which sprays were resorted to after the appearance of the disease no other cases appeared. This may be merely coincidental, but when one discovers that also following the adoption of sprays the total sick rate falls, especially that due to respiratory diseases, and bears in mind the current conception that the meningococci inhabit the nasal passages, one comes to have a very healthy respect for dichloramine-T as an agent for the prevention of diseases of upper respiratory tract origin. The organization num-

bered 7 in the chart has had the lowest measles and pneumonia rate in Camp Greene and is the only one which has systematically used the nasal spray. Its record is striking and forms a reasonable basis upon which to recommend that the routine use of nasal spraying with dichloramine-T be introduced into the camps for the prophylaxis of respiratory diseases.

THE INTRAVENOUS USE OF RED MERCURIC IODIDE

By L. W. Rowe, M.S., Detroit, Mich.

THE value of mercury in combating spirochetal diseases, particularly syphilis, has long been recognized, but its exhibition in the body in a satisfactory form is attended by many difficulties. The insolubility of many of the salts of mercury coupled with their toxic action upon body cells has rendered them of very little therapeutic value. The bichloride of mercury because of its comparatively greater solubility and consequent effectiveness has been used wherever possible, but its very corrosive action upon body tissues when administered hypodermically, or even upon mucous membranes when applied in a more concentrated solution, have greatly limited its use. In combination with blood serum the bichloride has been used intraspinally with some success, but the amount of bichloride which combines with the serum is not very great and a large dose is therefore necessary. The intravenous use of a satisfactory solution of the bichloride is impossible because of the great danger from embolism due to coagulation of some of the proteins of the blood.

The red iodide of mercury, HgI_2 (sometimes called biniodide) is as nearly insoluble in water as any of the salts of mercury, and for that reason is of no therapeutic importance. When combined with an equal amount of potassium iodide, a soluble compound is formed which is a very effective germicide, indicating that the characteristic action of mercury is exhibited. The efficiency of bichloride as a germicide is very great as is indicated by the fact that its germicidal coefficient is approximately one thousand (1000), or in other words that it is about one thousand times as efficient as pure carbolic acid, yet the average results, reported by various workers, of tests of solutions of mercuric iodide in potassium iodide show that this combination is fully five times as efficient as the bichloride, or 5000 times as efficient as pure carbolic acid. With this fact in mind it was thought advisable to attempt to obtain some idea concerning the possibilities of the use of mercuric iodide intravenously by determining the toxicity of this compound to animals when administered intravenously.

A search of the literature shows that Stassano and Gompel[1] have published several short articles in which they have commented upon the great germicidal activity and comparatively low toxicity of mercuric iodide and potassium iodide, but their experimental data has been largely concerned with the germicidal action and no definite comparisons could be made because of the lack of toxicity data.

*From the Research Laboratory, Parke, Davis & Co., Detroit, Mich.

[1]Stassano and Gompel: Compt. rend. Soc. de biol., 1913, lxxv, 42-44; Ibid., 1914, lxxvii, 9-11; Compt. rend. Acad. d. Sc., 1914, clviii, 1716-19.

Lydston[2] in a very short report of some clinical results states that he has frequently administered red mercuric iodide dissolved in potassium iodide intravenously with no harmful effects.

The tests shown in the accompanying tables were carried out upon guinea pigs, dogs, and rabbits, in order to show the toxicity of red mercuric iodide and potassium iodide when given intravenously to animals. Two comparative tests of mercuric chloride were also made.

TABLE I

TOXICITY OF RED MERCURIC IODIDE DISSOLVED IN POTASSIUM IODIDE ADMINISTERED INTRAVENOUSLY TO DOGS

DOG NO.	WEIGHT IN KG.	DOSE IN GM. PER KG.	DIL.	TOTAL DOSE IN C.C.	RESULT	REMARKS
1	8	.015	1:100	12	Died	Dead in 2 hours
2	6.5	.010	1:20	1.3	Died	Dead in 20 hours
3	7.5	.008	1:20	1.2	Died	Dead in 2 days
4	9.5	.005	1:30	1.43	Died	Dead in 5 days
5	7.	.004	1:50	1.40	Died	Dead in 2½ days
6	9	.004	1:20	.72	Died	Dead in 5 days
7	9	.0035	1:20	.63	Lived	Lived 3 weeks +
8	10.5	.0030	1:20	.63	Lived	Lived 3 weeks +
9	11.	.0025	1:20	.55	Lived	Lived 3 weeks +

M. L. D. .0040 gm. per kg. wt. of dog.

TABLE II

TOXICITY OF RED MERCURIC IODIDE DISSOLVED IN POTASSIUM IODIDE ADMINISTERED INTRAVENOUSLY TO GUINEA PIGS

NO.	WEIGHT IN GM.	DOSE IN GM PER KG.	DIL.	DOSE IN C.C.	RESULT	REMARKS
1	375	.008	1:100	.30	Died	Died during night
2	395	.005		.20	Died	Died during night
3	477	.004	1:1000	1.91	Died	
4	325	.004	1:500	.65	Died	
5	315	.004		.63	Died	
6	405	.004		.81	Died	
7	359	.004		.72	Died	
8	395	.0035		.70	Died	
9	458	.0035		.80	Died	
10	478	.0035		.83	Died	
11	455	.0035		.80	Died	
12	452	.0035		.79	Died	Died 6 days after dosing
13	445	.0030		.66	Died	
14	507	.0030		.76	Died	
15	519	.0030		.78	Died	
16	519	.0030		.78	Lived	Lived 10 days +
17	436	.0030		.65	Died	
18	463	.0025		.58	Lived	Lived 10 days +
19	327	.0025	1:1000	.82	Lived	" " "
20	350	.0020		.70	Lived	" " "
21	488	.0020	1:500	.49	Lived	" " "
22	344	.0015	1:2000	1.00	Lived	" " "
23	375	.0010		.75	Lived	" " "
24	373	.0008	1:5000	1.50	Lived	" " "

M. L. D. is .0030 gm. per kg. body weight.

[2]Lydston, G. F.: Jour. Am. Med. Assn., 1916, lxvii, 1446.

TABLE III

TOXICITY OF RED MERCURIC IODIDE DISSOLVED IN POTASSIUM IODIDE ADMINISTERED INTRAVENOUSLY TO RABBITS

NO.	WEIGHT IN GM.	DOSE IN GM. PER KG.	DIL.	DOSE IN C.C.	RESULT	REMARKS
1	2000	.025	1:100	5.0	Died	Dead in 1½ hours
2	1675	.015		2.51	Died	Dead in 3 days
3	1620	.010	1:50	.81	Died	Died during night
4	3150	.0080	1:100	2.52	Died	Dead in 1 week
5	1783	.0060	1:80	.86	Died	Dead in 1 week
6	2000	.0050	1:100	1.00	Lived	Lived 2 weeks +

TABLE IV

TOXICITY OF MERCURIC CHLORIDE ADMINISTERED INTRAVENOUSLY TO DOGS

NO.	WEIGHT IN KG.	DOSE IN GM. PER KG.	DIL.	DOSE IN C.C.	RESULT	REMARKS
1	6	.008	1:50	2.40	Died	Died in a few hours
2	7	6		2.10	Died	Died in a few hours
3	10	5		2.50	Died	Dead in 1 week
4	9	4		1.80	Lived	Lived two weeks

M. L. D. .005 gm. per kg.

TABLE V

TOXICITY OF MERCURIC CHLORIDE ADMINISTERED INTRAVENOUSLY TO GUINEA PIGS

NO.	WEIGHT IN GM.	DOSE IN GM. PER KG.	DIL.	DOSE IN C.C.	RESULT	REMARKS
1	455	.008	1:200	.73	Died	Died during night
2	493	.007		.69	Died	Died during night
3	495	.006		.59	Died	Died during night
4	572	.005		.57	Died	Died during night
5	446	.004	1:400	.72	Died	Died during night
6	451	.003		.54	Died	Died within 2 days
7	286	.0025		.72	Died	Died 3 days later
8	252	.0025		.63	Died	Died 2 days later
9	286	.0020	1:1000	.57	Lived	Observed for 1 week
10	360	.0020		.72	Died	Died in 4 days
11	358	.0020		.72	Died	Died in 3 days
12	327	.0015		.49	Lived	Observed for 1 week
13	368	.0015		.55	Lived	Observed for 1 week
14	395	.0015		.59	Lived	Observed for 1 week
15	318	.0010		.32	Lived	Observed for 1 week

M. L. D. is .0020 gm. per kg.

From data available in this laboratory the toxicity of phenol (carbolic acid) administered intravenously to guinea pigs is 0.2 gm. per kg. body weight. With this additional data Table VI of comparative toxicity and efficiency should be of value.

From Table VI the advantage of the use of either mercuric chloride or red mercuric iodide over the use of phenol, by comparison of toxicity and germicidal efficiency, can be readily seen. The figures in parenthesis indicate the comparison between mercuric chloride and mercuric iodide alone, using the figures obtained for mercuric chloride as unity. These figures show that although red mercuric iodide in potassium iodide when given intravenously is very little

TABLE VI

COMPARISON OF TOXICITY AND GERMICIDAL EFFICIENCY OF PHENOL, MERCURIC CHLORIDE, AND MERCURIC IODIDE

	GERMICIDAL COEFFICIENT	TOXICITY INTRAVENOUSLY TO GUINEA PIGS	TOXICITY INTRAVENOUSLY TO DOGS
Phenol	1	1	no data
Mercuric chloride	1000 (1)	100 (1)	.005 gm. per kg. (1)
Mercuric iodide in KI	5000 (5)	66⅔ (⅔)	.004 gm. per kg. (1¼)

if any more toxic (in the case of guinea pigs it is less toxic) than mercuric chloride, yet its germicidal efficiency is five times as great as that of mercuric chloride. It should consequently be greatly preferred to the bichloride if only because of its greater efficiency.

Another factor which is worthy of serious consideration is that in all the intravenous injections into animals of mercuric iodide (some of the injections being made rapidly) not one of the animals died suddenly following the injection. This would indicate that a solution of red mercuric iodide in potassium iodide has no very strong tendency to coagulate any of the constituents of the blood when introduced directly into the blood stream and thereby cause sudden death from embolism. On the other hand according to our observations, solutions of mercuric *chloride* have several times produced sudden death when administered intravenously to animals, indicating that embolism is often formed. This is very apt to happen if the injection is made rapidly.

Solutions of mercuric iodide like those of most mercury salts cause marked local irritation when administered subcutaneously or intramuscularly, so that the iodide will probably never become very popular for such methods of administration.

However, with the increase of our use of intravenous therapy because of advantages which it possesses in many instances and particularly because the most successful method of combating a severe systemic disease such as syphilis is by intravenous injection of such agents as salvarsan, etc., in solution, it seemed advisable to contribute the above tables of data to our knowledge of the pharmacologic action of such an important germicide as red mercuric iodide.

It is not the object of this paper to assure physicians of the safety of the intravenous use of this salt of mercury because this can not be assumed from animal experimentation alone. It is intended merely to point out certain therapeutic possibilities as indicated by animal tests. If these can be substantiated by carefully conducted clinical tests, as it is hoped they can be, the purpose of the article will have been attained.

SUMMARY

Red mercuric iodide in combination with an equal amount of potassium iodide can be injected in solution into animals intravenously with comparative safety if reasonable care is exercised in the manner of injection and in the size of the dose injected.

It is very little if any more toxic than mercuric chloride, safer for intravenous use, and because of its greater germicidal efficiency should be found to be of therapeutic value.

AN OUTLINE FOR THE COMBINED TEACHING OF PATHOLOGY AND BACTERIOLOGY IN SMALL MEDICAL COLLEGES*

By Ellis Kellert, M.D., Albany, N. Y.

At no previous time in the history of American medical institutions has there been so great a demand for conservation of resources, concentration of effort, or emphasis of fundamental principles. The withdrawal of a large portion of the teaching and scientific staffs of all medical colleges for military service and the consequent reduction in the number of available assistants for teaching purposes calls for a rearrangement of courses and methods. This is particularly true of small medical colleges where the number of instructors barely suffice in normal times to carry on the work. For these reasons, and as the result of a successful experience of three years' duration, the following outline is offered as a suggestion in the conduct of classes in pathology and bacteriology.

It is needless to say that the student who is not thoroughly instructed in histology, physiology, and biochemistry has been poorly prepared for the study of pathology. The inability to visualize normal structures and functions makes it impossible to recognize and interpret abnormal changes, and, therefore, the essential conceptions of disease may be lacking. The structure of a lesion will have but little significance for the student not acquainted with histologic anatomy, and there must be developed in him the power to identify the cause and effect, and predict the remote result of the changes which he observes. With training in the recognition of isolated lesions will come the ability to conceive the general bodily changes. With these requirements in mind the instructor must plan to utilize his time and facilities to greatest advantage. The essentials are to be emphasized and the purely philosophic phases of the subject omitted. The didactic teaching should be reduced to a minimum and experimental demonstrations made as numerous as possible. The outline is also suggested at this time because many men without previous teaching experience have been suddenly called upon to conduct courses in pathology and bacteriology.

The tendency at present in medical schools is toward the "block" system of teaching the laboratory courses. The advantages of this system are that the student devotes himself entirely to the one subject which usually occupies half the school year. The teacher is free, therefore, for study or research during the remaining months. The chief disadvantages are the rapidity with which progress must be made and the possible lack of sufficient material during the brief period of the pathologic course. Great care must be exercised to avoid bewildering the student as the subject expands and the method here

*From the Bender Hygienic Laboratory, Albany, N. Y.

suggested will accomplish much toward giving him a comprehensive view of the studies of pathology and bacteriology.

The work includes the following subjects: general and organal pathology, bacteriology, animal parasitology, tumors, and serology. The course is begun with exercises in bacteriologic technic. The classification of microorganisms is then studied, after which the student acquaints himself with the physiologic characteristics of bacteria. He then proceeds to the study of inflammation and the retrograde changes in tissues. The specific pathogenic bacteria are next investigated, first morphologically and culturally, immediately after which the lesions they induce are studied. There is thus given a complete picture of cause and effect and the association of a certain organism with a characteristic lesion is firmly fixed in the student's mind. Fresh tissues or preserved specimens from which microscopic sections have been made are exhibited and in his attempts at differential diagnosis, the student makes use of the freezing microtone or resorts to animal inoculation. By this method, fatigue from continuous concentration in one subject is avoided and interest is sustained by retaining the subject in its entirety. Thus by way of example, the bacteriology of the typhoid bacillus may be studied in the morning session and in the afternoon, the pathologic anatomy of typhoid fever. The teaching effort is reduced to a minimum, and the student gains a more comprehensive knowledge of the subject. After progress has been made, protocols of actual autopsies are read and discussed by the class in conference. The organs are exhibited, changes noted, the anatomic diagnoses tabulated and the lesions explained.

Although the above would seem to be sufficient to keep the student quite busy, yet he is found to have unoccupied time especially during the last month or six weeks when relieved of the extensive bacteriologic technic. Each student, therefore, is assigned a problem for investigation, which, except in the case of unusually capable students, should not be original, in order that a comparison may be made with the results of a previous investigation. If desired however, original problems may be undertaken and varied according to the mental capacity and technical ability of the individual student. Thus one man may be given the problem of determining the chief varieties of bacteria in the throats of twenty normal adults, another may attempt to isolate typhoid bacilli from the urine and stools, another will determine the antiseptic properties of certain chemicals, and still another study the bacteria found on hair brushes. Numberless other topics will suggest themselves. Pathologic studies involving animal inoculations may also be suggested, but such problems demand too much of the teacher's time and there is needless sacrifice of animal life. Experiments with the larger animals are best reserved for class demonstrations. The object of each study should be systematic work, minute observation, recording of data and careful technic. When the problem is finished, the paper is read before the class and discussed.

Lectures should be few, but demonstrations numerous. At the end of the half day's session a meeting of the class is held and the subject of the day discussed in conference. Great interest is evinced by students in going over an

autopsy protocol, for they soon learn that this form of study is stimulating; not only with reference to the work in hand, but also to related subjects. The method in reality is case teaching, and, when gross and microscopic material can be exhibited, is the ideal procedure.

In the outline which follows no headings are included for diseases of the nervous system*, but these may be inserted at convenient points and new headings created such as diseases of the meninges, atrophic and degenerative sclerosis of the cord, of the brain-stem, cerebral hemorrhage, cerebral softening and others which will suggest themselves.

OUTLINE OF COURSE

Historical—classification.
Bacteriologic methods.
  Staining.
  Physiology of bacteria.
  Destruction of bacteria.
    Carbolic coefficient.
Blood—histological review.
    Theories of immunity.
Inflammation.
Pyogenic cocci.
Retrograde processes.
Streptococcus—pneumococcus group.
Gonococcus—meningococcus group.
Pneumobacillus.
Pneumonia.
B. mallei.
Glanders.
Hemoglobinophilic bacteria.
    Whooping cough.
    Influenza.
B. pyocyaneus group.
B. anthracis.
    Anthrax.
Colon—typhoid group.
    Dysentery.
    Typhoid fever.
B. diphtheriæ.
    Diphtheria.
    Pseudo-diphtheria bacilli.
Acid-fast bacilli.
  Tuberculosis.
  Leprosy.
B. pestis.
  Plague.
The Anaerobic bacilli.
  Anaerobic methods.
  B. aerogenes capsulatus.
  B. of malignant edema.
    Gaseous gangrene.
  B. tetani.
    Tetanus.
Pathogenic spirillæ.
    Cholera.
    Relapsing fever.
    Vincent's angina.
Treponema pallidum.
    Syphilis.
    Typhus.
Poliomyelitis.
Immunity.
Phagocytosis.
Opsonins.
Agglutinins.
Precipitins.
Vaccines.
Serums.
Anaphylaxis.
Serologic methods of diagnosis.
Tumors—
  Benign connective tissue tumors.
  Malignant connective tissue tumors.
  Endothelioma.
  Myoma.
  Glioma.
  Neuroma.
  Benign epithelial tumors.
  Malignant epithelial tumors.
  Mixed tumors.
  Teratoid tumors.
  Cysts.
Animal parasites.
  Protozoa.
  Amebæ.
  Flagellates.
  Ciliates.
  Amebic dysentery.
Sporozoa.
  Coccidia.
  Coccidiosis.
Hemosporidia.
  Malaria.
Sarcosporidia.
Vermes.
  Trematodes.
  Cestodes.
  Nematodes.
Arthropoda.
Ductless glands.
Pernicious anaemia.
Leukemia.
Hodgkin's disease.
Spleen and lymph nodes.
Circulatory system.
Lungs.

*An extensive course in diseases of the nervous system was given by the department of neurology.

OUTLINE OF COURSE—CONT'D.

The Higher forms of microorganisms.
- Actinomycosis.
- Sporotrichosis.
- Thrush.
- Ringworm.
- Favus.

Filterable viruses.
Diseases of unknown etiology.
Rabies.
Stomach and intestine.
Pancreas.
Liver.
Genitourinary system.
Female genitalia.
Male genitalia.

The outline simply indicates the general headings and should be amplified by the instructor by the addition of numerous subheadings. Because of present-day interest, such topics as measles, meningitis, pneumonia, gangrene, vaccination, and venereal diseases should be treated in great detail.

Owing to the abundance of surgical material in most laboratories, surgical pathology is prone to receive undue importance and the teaching may degenerate into the gross and microscopic examination of specimens, thus making the course a diagnostic one instead of a biologic study. This tendency is also present in clinical teaching laboratories in hospitals where the examination of blood and excreta receive undue consideration. Under such circumstances the student tends to become a technician and the intellectual study of medicine may be neglected. During the fourth year, a course in advanced pathology should be provided. In the light of his brief clinical experience, such study will be of great value to the student and should include a thorough consideration of functional pathology.

In most schools too little time is devoted to animal parasitology. This phase of comparative pathology is extremely interesting and instructive, elucidating principles of pathology not otherwise so readily attained. The lesions are pronounced, material abundant, and the injurious agents so easily procured that the subject should receive greater consideration. No other branch of pathology illustrates so well the relation between cause and effect. In fact, the study of animal parasites would at the present time, when such keen interest is manifested by the general public in hygiene and disease, be an exceedingly profitable subject for courses in biology. This plan has been suggested on a previous occasion by Dr. Theobald Smith and might well be adopted in premedical studies. The interest thus aroused in animal parasitology may result in the more active pursuit of comparative pathology.

The outline above presented is intended for small schools. There are those who feel that the subjects of pathology, bacteriology, and serology are sufficiently comprehensive to be conducted under separate departments. Where this is done, much overlapping and repetition occur and it is not advisable unless extensive resources, financial and otherwise, are available. Our experience has been that two instructors and one technician can readily conduct the above combined courses, omitting nothing considered necessary in teaching the principles of pathology and bacteriology. That changes in the methods of teaching these subjects are gradually coming about is indicated by such recent works on pathology by Mallory and also by MacCallum. It would be a serious error, indeed, to have the student regard these subjects in any other light than as one science.

LABORATORY METHODS

A CLINICAL METHOD FOR DETERMINING THE RESPIRATORY EXCHANGE IN MAN*

By R. G. Pearce, B.A., M.D., Cleveland, Ohio

SINCE the determination of the respiratory exchange in man is of some importance in the study of certain diseases of respiration, circulation, and metabolism, and also because directions for carrying out the necessary procedures are not generally available, I have thought it might be af assistance to present here brief directions for the Tissot[1] and the Douglas methods.[2] These methods have been found to compare favorably in accuracy with others in use at present,[3] and because of their adaptability and simplicity they are specially suited for clinical work.

By these methods the energy metabolism of the body is calculated from

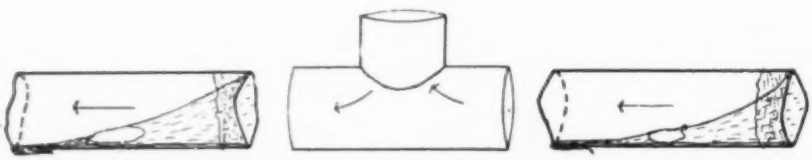

Fig. 1.—Valves used by the author.

oxygen consumption or carbon-dioxide excretion per minute (indirect calorimetry), the figures for which are determined from the minute volume and percentile gaseous composition of the expired air.

The subject breathes through valves which automatically partition the inspired and the expired air. The expiration from a number of respirations are collected in a spirometer or bag, and the volume of the respirations per minute is determined. The gaseous composition of the expired air is determined by gas analysis, and the oxygen consumption and energy output of the body are calculated from the data obtained.

DESCRIPTION AND USE OF PARTS OF THE APPARATUS[1]

The Mouthpiece and Valves.†—The mouthpiece is made of soft pure gum rubber, and consists of an elliptical rubber flange having a hole in the center 2 cm. in diameter, to which on one side a short rubber tube is attached. On the opposite side of the hole, at right angles to the rubber flange, are attached two rubber lugs. The rubber flange is placed between the lips, and the lugs are

*From the Cardiorespiratory Laboratory, Medical Service, The Lakeside Hospital, Cleveland, Ohio.

†The mouthpiece, nose clip, face mask, Douglas bag, and gas-analysis apparatus can be secured from H. N. Elmer, 1140 Monadnock Building, Chicago, Ill.

held by the teeth. The rubber tube of the mouthpiece is connected to the tube carrying the valves. The nose must be tightly closed if mouth-breathing is used. This is accomplished by a nose clip, which consists of a V-shaped metal spring, the ends of which are provided with felt pads. A toothed rachet is attached to the ends of the spring, and serves to hold the spring tightly clamped on the nostrils in the proper position (see Fig. 2).

Some individuals experience great distress when made to breathe through the mouth. For these it is best to use a face mask. Unfortunately at the present time no mask is entirely satisfactory. Perhaps the best is one sold by Siebe,

Fig. 2.—*A*, Nose clip; *B*, Face mask; *C*, Mouthpiece.

Gorman & Co., which is pictured in the illustration. After being placed in position the face mask should be tested for leaks, which can be done by putting soap around the edges.

The Valves.—The valves of Tissot are probably the best for the purpose, but they are expensive and hard to obtain. We have made perfectly satisfactory valves from the prepared casings used in the manufacture of Bologna sausage.[4] These can be obtained preserved in salt, and they will keep indefinitely on ice. When needed a short piece is taken, washed free from salt by allowing water from the tap to run through it, and softened in a weak glycerin solution. The

gut becomes very soft and pliable, and does not dry quickly. A piece of the casing about 10 cm. long is threaded through a glass tube of about 15 mm. bore and 4 to 6 cm. long. One end of the casing is brought around the outside of the tubing and secured by means of a thread. The lower end of the membrane is pinched off and the casing is then cut a little more than half way across its middle, so that this opening will lie just within the free end of the tube when the casing

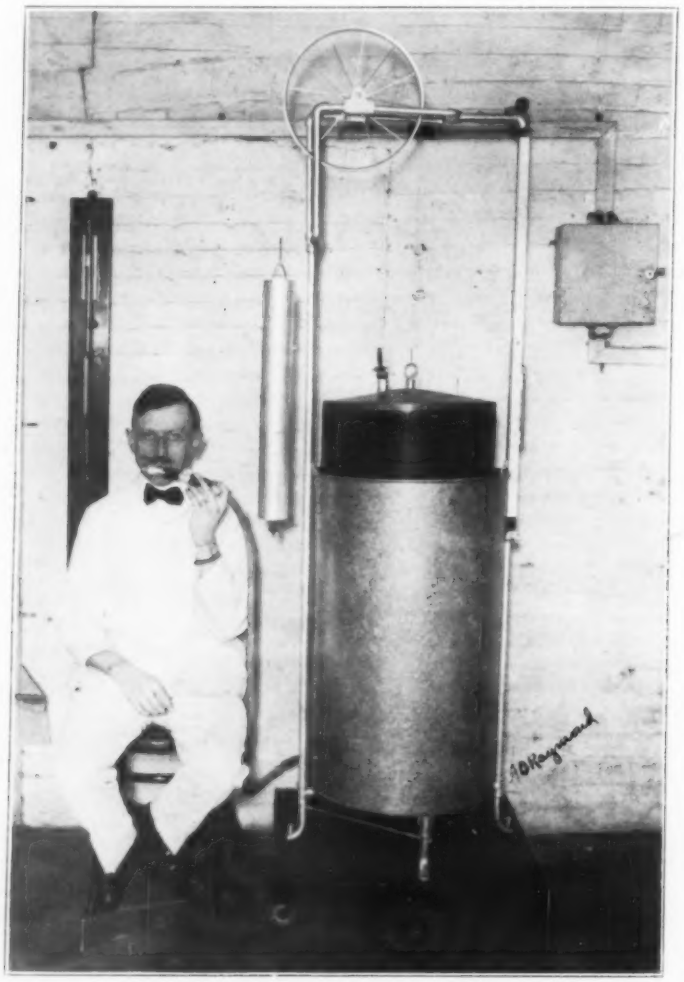

Fig. 3.—The Tissot spirometer. In actual experiment, subject is reclining or lying down and the valves and mouthpiece are held with a clamp.

is drawn back through it. The loose end of the casing is twisted slightly—an essential procedure,—and is then secured by a thread on the outer side of the tube. If properly made, the valve will work freely without vibration, and the opening will be sufficiently large to allow a good current of air to pass. It should collapse instantly and be air-tight when the current of air is reversed. The back lash, or lag of closure, of these valves is extremely small, and they will open or close with a pressure of air not exceeding the pressure changes in

normal respiration. When not in use, the valves should be kept in glycerin water on ice. Valves prepared in this way have been in use a month without loss of efficiency. They are, however, made with so great ease that new valves are provided for each subject, and they are, therefore, especially adapted to ward work.

These valves are inserted in reverse order into a supporting metal T-piece, and the joints made air-tight by tape. The stem of the T is connected with the mouthpiece. Through a rubber tube of about ¾-inch bore, the expired air is collected in the spirometer.

The Tissot Spirometer.—The Tissot spirometer is pictured in Fig. 3. We have found the 100-liter size to be very serviceable in the clinic. This instrument is mounted on a platform having rubber wheels, and can be moved about the wards with ease. The bell of the spirometer is made of aluminum and is suspended in a water-bath between the double walls of a hollow cylinder made of galvanized iron.* The height of the bell is 72 cm., and the diameter 42 cm. An opening at the bottom of the cylinder connects through a three-way stopcock with the rubber tube leading from the expiratory valve of the mouthpiece. The bell is counterpoised by means of a weight. In the original Tissot spirometer an automatic adjustment permitted water in amount equal to the water displaced by the bell to flow from the spirometer cylinder into the counterpoise cylinder as the bell ascended out of the water. The bell, being heavier out of water than when it is immersed, is accordingly counterpoised in any position, although Carpenter[3] has shown that this refinement is unnecessary. An opening in the top of the spirometer permits the insertion of a rubber stopper, through which are inserted a thermometer, a water manometer, and a stopcock with tube for drawing the sample of air. A scale on the side of the instrument gives the volume of the air.

During an observation the subject sits in a reclining position or lies upon a couch. When the bell of the spirometer is placed at zero, the mouthpiece adjusted in the mouth, and the nose clamped, respiration is started, the expirations being passed through the stopcock, which is so turned as to allow them to pass to the outside air. After a few minutes the stopcock is turned so that the expirations are passed into the spirometer for a definite length of time. At the end of the period the cock is again turned, and after the barometric pressure, temperature, and volume of the air have been noted, the composition of the air is determined.

The Douglas Bag.[2]—This is made of rubber-lined cloth, and is capable of holding from 50 to 100 liters. It is fitted with straps and is easily carried on the back. In making observations the bag is placed on the shoulders and connected with the valves, the mouthpiece of which is placed in the mouth. Respirations are then commenced with the three-way valve turned so as to allow the expirations to pass directly into the outside air. After respiratory equilibrium is established, the three-way valve is turned during an inspiratory period so that the succeeding expirations may pass into the bag. The time required to fill the bag comfortably is determined with a stopwatch. The air which has

*The instruments in use in my laboratory were made by the Cleveland Metal Products Company, Cleveland, to which we wish to extend our thanks.

been collected in the bag during the period is thoroughly mixed and passed through a meter, the temperature and barometric pressure are noted, and a sample analyzed on the Haldane gas apparatus. The bag should be emptied completely by rolling it up when nearly empty, and then allowed to fall back naturally before and after using.

The Haldane Gas-analysis Apparatus.—The Haldane method of analysis of expired air is simple and easily learned. The apparatus (Fig. 5) consists of a gas burette, a control burette of the same size (both surrounded with a water jacket), and bulbs containing dilute caustic potash soda solution for the absorption of the carbon dioxide and an alkaline pyrogallate solution for the ab-

Fig. 4.—The Douglas bag method for determining the respiratory exchange. The arrangement of mouthpiece, valves, and connecting tubes have been found to be more convenient than that recommended by Douglas.

sorption of the oxygen. The gas burette is connected with the bulbs by a two-way stopcock, which allows a sample of gas to pass into either bulb. The control tube is put into connection with the burette through a manometer tube, which is connected with the alkali bulb, and can be made to compensate for any changes in temperature that may occur during the course of the analysis. For an analysis the gas is transferred to the burette from the sampling tube, saturated with water vapor over mercury, and then measured, after which it is transferred into the caustic soda solution to free it from carbon dioxide, and then returned to the burette to determine the loss of volume due to carbon-dioxide absorption. It is then transferred into the alkaline pyrogallate solution,

which frees it from oxygen, after which it is again brought back to the burette to determine the loss in volume due to the absorption of the oxygen.

The detail of the Haldane apparatus is shown in Fig. 5. The measuring burette holds 21 c.c. The bulb is of 15 c.c. capacity, and the graduated stem,

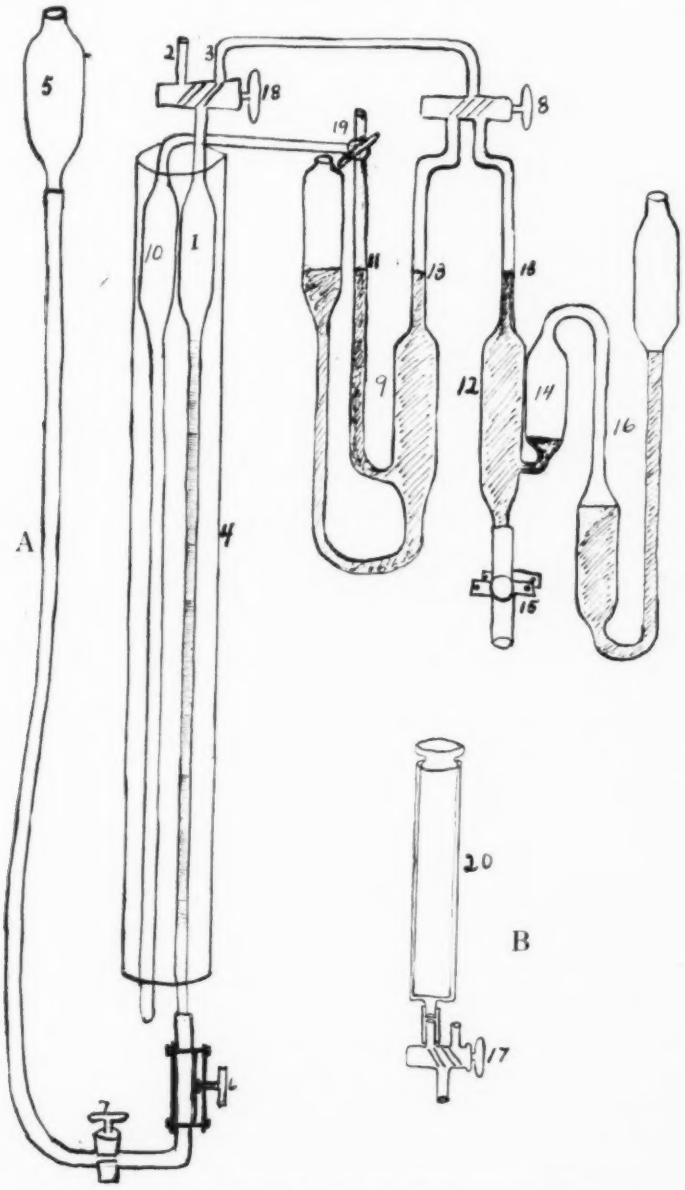

Fig. 5.—Haldane gas apparatus (*A*) and Pearce sampling tube (*B*).

which is about 4 mm. in bore and 60 cm. in length, is graduated to 0.01 c.c. from 15 c.c. to 21 c.c. The stopcock at the top of the burette is double-bored, so that in one position air can be drawn in from a gas sampler (*2*) and in another sent into the absorption bulbs (*3*). The lower part of the burette extends through

the rubber cork at the bottom of the water jacket (*4*). A piece of rubber tubing is attached to the bottom of the burette and placed within a metal tube, inside of which is a piece of metal which presses against the rubber tubing, the pressure being controlled by means of a fine adjusting screw (*6*). Below this a glass stopcock (*7*) connects with rubber tubing to the mercury leveling bulb (*5*). The absorption bulb for carbon dioxide, containing 20 per cent NaOH or KOH (*9*), is put in connection with the burette by suitably turning stopcocks (*3* and *8*).* The control burette (*10*) is also in connection with this bulb through the manometer tube (*11*).† Any variation in temperature which may occur during the analysis will cause the level of the alkaline solution in the manometer to change.

When final readings of the shrinkage of volume are made, the level of the caustic solution is returned to the level of that in the manometer. By so doing, any error due to temperature changes is avoided, since change in temperature must be equal in the two burettes.

The absorption bulb for oxygen (*12*) is filled with a solution made by dissolving 10 grams of pyrogallic acid in 100 c.c. of a nearly saturated KOH solution. The specific gravity of the KOH should be 1.55, which is obtained approximately by dissolving the sticks (pure by alcohol) in an equal weight of water. The mark (*13*) on the stem of the bulb indicates the level at which the solutions should stand. Enough pyrogallate solution is introduced through tube *15* to fill bulbs *12* and *14* two-thirds full, then pyrogallate solution is poured into tube *16* until the difference in level of the fluids is sufficient to produce enough pressure to raise the level of the pyrogallate solution in *12* to the level *13* on the stem. Stopcock *8* must be open during this procedure. It may be necessary to add or take away a little pyrogallate solution through *15* to attain the above level.

Care must be taken to allow for complete absorption of oxygen from the air that is entrapped between *14* and *16* before an analysis is made; otherwise changes will be produced in the level of the pyrogallate solution. The air in the capillary tubing connecting the burettes with the absorption bulbs must also be freed of carbon dioxide and oxygen. This can be accomplished by making a dummy analysis of atmospheric air before the real analysis. Great care must be taken to have atmospheric pressure in all the tubes at the start of the analysis. This is accomplished by opening the stopcock in the burette first to atmospheric air and then to the absorption bulbs, until no further change in the level of the fluids in the stems of the absorption bulbs occurs. This level is then marked and used as the standard. A small amount of water in the burette over the mercury assures saturation of the air with water vapor. Time for drainage must be allowed before making readings.

A very serviceable *sample tube* for the transfer of air can be made from a 30 c.c. ground-glass syringe, to which is attached a two-way stopcock. A cut

*The stopcock (*8*) is double-bored, so that the bulb leading from the burette can be brought into connection with either *9* or *12*.

†This tube also has a three-way stopcock (*19*), so that the tube may be opened to the outside.

of this is shown in the illustration. The dead space in these syringes is washed out by working the piston back and forth several times. A thin coating of vaseline prevents leakage of the gas. I have found that these sampling tubes will retain a sample of expired air without change up to eight hours.

Manipulation of Apparatus.—The sampling syringe (*20*) is attached to opening *2* of burette, and its stopcock (*17*) opened to atmospheric air as represented in the illustration. The level of the mercury is then brought to the level of the stopcock in the syringe and the stopcock is closed. The bulb of mercury is lowered so that the mercury falls in the burette. This draws the piston of the syringe with it, and fills the burette with air from the syringe. It is advisable to put a little positive pressure on the piston of the syringe in the maneuver to prevent possible leakage. When all of the air is in the burette, a slight positive pressure is produced in the burette by gently pressing on the piston, and immediately thereafter the stopcock on the syringe (*17*) is again turned to the original position. This allows the pressure of air in the burette to come to that of the atmosphere pressure. The height of the mercury is now adjusted to a convenient height in the burette by closing cock *7* and turning the milled screw *6*. The cock *18* is now made to communicate with the absorption bulbs. If the air in the burette is at atmospheric pressure, no change will occur in the level of the fluids. The reading is then taken on the burette.

The next step in the analysis consists in turning stopcock *8* to communicate with the caustic soda solution in bulb *9*, and the leveling tube *5* is raised, forcing mercury into the burette and the air into bulb *9*. The gas is passed back and forth several times until absorption is complete, as can be determined by the fact that the level of the mercury in the burette remains constant when the fluid in the bulb is returned to its original level (*13*) on the stem. In this adjustment it is convenient to make the gross leveling by the mercury bulb and the fine leveling by closing *7* and turning *6* until the fluid in *9* is at the original height. The reading on the burette indicates the loss in volume due to the carbon dioxide absorbed.

The oxygen is removed by a similar procedure, the gas being passed into the alkaline pyrogallate solution by turning cock *8* to communicate with bulb *12*. The absorption of oxygen is slower than for carbon dioxide, and more care must be taken to get complete absorption. The air in the capillary tubing between the fluid in *9* and stopcock *8* must be washed out several times in order to get the oxygen which is left in it after the absorption of the carbon dioxide. When this is complete, the final reading on the burette is made and the loss in volume from the second reading represents the oxygen.

THE CALCULATIONS

The calculation of the percentile composition of the air and of the respiratory quotient is represented in the following example of an actual analysis.

The temperature and barometric pressure as taken at the time of the experiment were 20° C. and 747 mm. Hg.

CO_2 analysis—

1st reading of burette	20.00
2nd reading of burette after absorption of CO_2	19.20
CO_2 absorbed	0.80

0.80 ÷ 20 = 4.0 per cent CO_2 in expired air.
Since there is 0.03% CO_2 in atmospheric air, 3.97% of the CO_2 in the expired air was derived from the blood.

O_2 analysis—

2nd reading of burette	19.20
3rd reading of burette after absorption of O_2	15.90
O_2 absorbed	3.30

3.30 ÷ 20 = 16.50% of O_2 in expired air.

Determination of R. Q.—

O_2 in atmospheric air =	20.94%
$O_2 + CO_2$ in expired air (16.50 + 4) =	20.50%

100 − 20.94 = 79.06%* N in atmospheric air.
100 − 20.50 = 79.50% N in expired air.

Since the nitrogen is not changed in volume, the last figure shows that more oxygen must have been taken in during inspiration than $O_2 + CO_2$ has been given back in expiration. This obviously must be taken into account in the calculations. The amount of O_2 actually inspired for each 100 c.c. of air expired is found as follows:

$$\frac{20.94 \text{ (percentage of } O_2 \text{ in atmospheric air)}}{79.06 \text{ (percentage of } N_2 \text{ in atmospheric air)}} \times 79.60 \text{ (percentage of } N_2 \text{ in expired air)};$$

or 0.265 (constant factor) x 79.5 (percentage N_2 found for this observation) = 21.07, the volume of O_2 which would have been present in expired air to account for N_2 present.†

21.07 − 16.50 = 4.57% O_2 actually absorbed.
3.97 = percentage CO_2 excreted.
$\frac{3.97}{4.57} = 0.87$, the respiratory quotient, or ratio of CO_2 excreted to O_2 absorbed.

Total Gas Exchange.—The volume of air expired in 15 minutes into the Tissot spirometer was found to be 100 liters measured at 20° C. and 747 mm. Hg. (brass-scale barometer). This volume of gas must be corrected so as to give the volume of dry air at 0° and 760 mm. Hg. To do this two things must be taken into account: (1) Since the expired air is saturated with water, the pressure due to water vapor must be subtracted from the observed barometric pressure to obtain the true pressure. The vapor tension of water for various temperatures is given in Table II. (2) The barometer tube lengthens or contracts with heat or cold, and, therefore, the barometric readings must be corrected. The corrections for ordinary barometric readings are found in

*This is the constant O_2 percentage in air.

†This calculation can be simplified by the use of Table I which gives the O_2 figure corresponding to the various percentages of $O_2 + CO_2$ in the expired air.

Table III. The figure corresponding to the temperatures is subtracted from the barometric reading in order to obtain correct barometric pressure.

In the above experiment, the correction for the barometer is 2.41 mm. (see Table III), and that for vapor tension at 20° C. is 17.4 (see Table II).

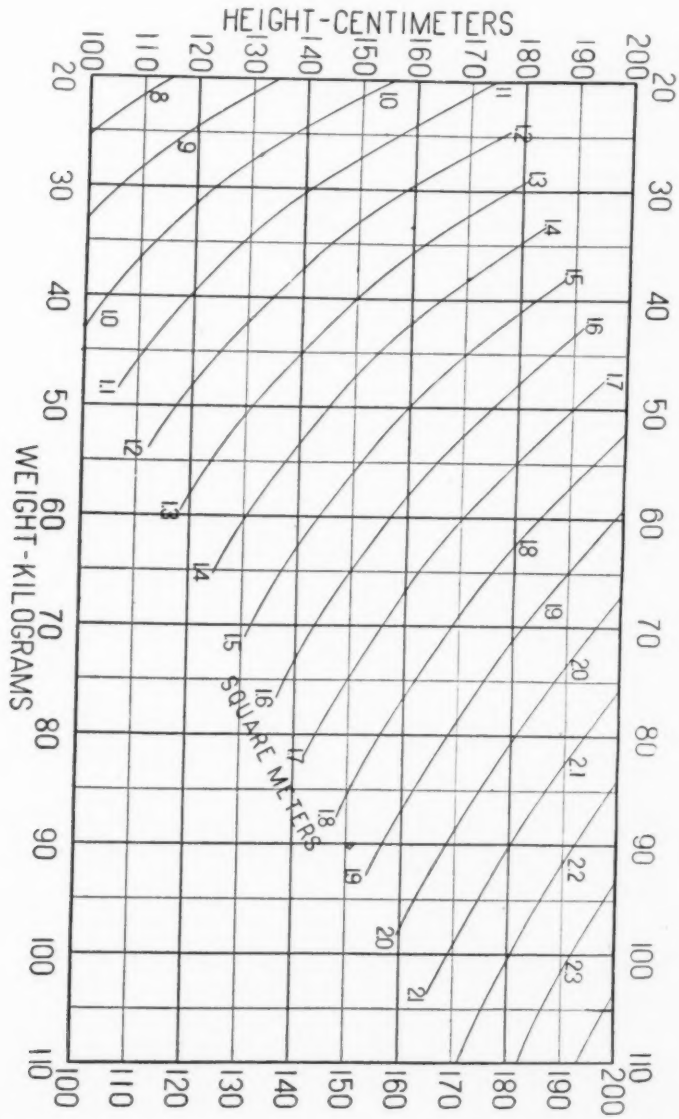

Fig. 6.—Chart for determining surface area of man in square meters from weight in kilograms (Wt.) and height in centimeters (Ht.) according to the formula: Area (Sq. Cm.) = Wt. 0.425 XHt. 0.725 X 71.84. (From Dubois & Dubois, Arch. Int. Med., 1917, vol. 17.)

Actual Barometric Pressure.—747 — (17.4 + 2.39) = 727.21 mm. The coefficient of expansion of gases is taken as 0.003665, or 1/273; therefore, the volume of 0° equals the volume at 1° divided by 1/0.003665 t; and hence

$Vo = \frac{V \times 273}{273 + t} = \frac{V}{1 + 0.003665\ t}$, when Vo = Volume at O° and V = Volume at t°.

The volume of gas being inversely as the pressure, $Vo = \frac{VP}{760}$, where V = volume at P pressure; or working both corrections together,

$$Vo = \frac{VP \times 273}{760 \times (273 + t)} = \frac{VP}{760\ (1 + 0.003665\ t)}.$$

This formula applied to the present problem reads:

$$VO = \frac{100 \times 727.2}{760\ (1 + 0.003665 \times 20)} = 89.2 \text{ liters.}$$

The latter calculation can be considerably simplified by using standard tables which give constants for corrections of gas volumes. These are easily obtainable and are given in part in Table IV. According to these tables, for 20° C. and 727.21 mm. Hg. B. P., the factor is 0.89124.

$0.89124 \times 100 = 89.124$ liters, 0° C. and 760 mm. Hg.
$0.89124 \times 4.57 = 46$ liters of O_2 in 15 min., or 18.4 liters per hour.

The Caloric Value Calculated from the Gas Exchange.—By reference to Table V, giving the heat value of 1 liter of O_2 at various respiratory quotients, it is found that at a R.Q. of 0.870, 4.888 calories are expended; 18.4 liters of O_2 is therefore equivalent to $18.4 \times 4.888 = 90$ calories.

The results must be calculated for surface area as well as body weight, since the metabolism is roughly proportionate to the surface area. Suppose the subject weighed 85 kg. and was 170 cm. in height; by reference to the chart for determining the surface area of man (Fig. 6), this is found to be 1.96 square meters. The caloric expenditure per square meter in the above case is therefore $\frac{90}{1.96} = 45.8$ calories.

TABLE I*

THE VOLUME PERCENTAGE OF OXYGEN WHICH WOULD BE PRESENT IN THE INSPIRED AIR TO ACCOUNT FOR THE $CO_2 + O_2$ FOUND IN THE EXPIRED AIR

$\%CO_2 + \%O_2$ IN EXPIRED AIR	VOL. % OF O_2 IN INSPIRED AIR	$\%CO_2 + \%O_2$ IN EXPIRED AIR	VOL. % OF O_2 IN INSPIRED AIR
19.4	21.38	20.4	21.10
19.5	21.35	20.5	21.07
19.6	21.31	20.6	21.04
19.7	21.28	20.7	21.01
19.8	21.25	20.8	20.98
19.9	21.22	20.9	20.96
20.0	21.20	21.0	20.93
20.1	21.18	21.1	20.90
20.2	21.15	21.2	20.88
20.3	21.13	21.3	20.86

*The table gives the oxygen in the inspired air equivalent to the sum of the percentages of CO_2 and O_2 in the expired air.

TABLE II*

TENSION OF AQUEOUS VAPOR IN MILLIMETERS OF MERCURY

Temp.	15°	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°
Mm.	12.7	13.5	14.4	15.4	16.3	17.4	18.5	19.7	20.9	22.2	23.5

*To obtain the dry barometric pressure, subtract the mm. Hg. corresponding to the temperature of the air from the barometric pressure at the time of the experiment.

TABLE III*

TEMPERATURE CORRECTIONS TO REDUCE READINGS OF A MERCURIAL BAROMETER WITH A BRASS SCALE TO 0° C.

Temp.	700 mm.	710 mm.	720 mm.	730 mm.	740 mm.	750 mm.	760 mm.	770 mm.
15°	1.69	1.72	1.74	1.77	1.79	1.81	1.84	1.86
20°	2.26	2.22	2.32	2.36	2.39	2.42	2.45	2.48
25°	2.83	2.87	2.91	2.95	2.99	3.03	3.07	3.11

*Subtract the appropriate quantity as found in table from the height of the barometer. The table is for a barometer with a brass scale, and the values are a little lower (about .2 mm.) than for the glass scale. The corrections for intermediate temperatures can be approximated.

TABLE IV*

TABLE FOR REDUCING GASEOUS VOLUMES TO NORMAL TEMPERATURE AND PRESSURE

Mm.	15°	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°
720	.898	.894	.891	.888	.885	.882	.880	.877	.873	.870	.867
730	.910	.907	.904	.901	.897	.894	.891	.888	.885	.882	.879
740	.922	.919	.916	.913	.910	.907	.904	.901	.897	.894	.891
750	.935	.932	.928	.925	.922	.919	.916	.913	.910	.907	.904
760	.947	.944	.941	.938	.934	.931	.928	.925	.922	.919	.916
770	.960	.957	.953	.950	.948	.945	.940	.936	.933	.930	.927

*The observed volume, when multiplied by the factor corresponding to the temperature and corrected pressure, will give the volume of the expired air reduced to 0° and 760 mm.

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A HIGHLY DIFFERENTIATING POLYCHROMATIC TOLUIDIN-BLUE STAIN*

By Moses Barron, M.D., Minneapolis, Minn.

ANYONE working in a clinical-pathologic laboratory realizes the need for reliable stains that possess the virtues of precise differentiating powers, as well as simplicity and rapidity of technic. The almost countless number of stains that go to make up the armamentarium of the laboratory technician testify to the continuous efforts of many workers towards discovering serviceable stains.

For many years, I have been impressed with the polychromatic qualities of toluidin-blue. I became acquainted with the dye as an ingredient of Ponder's stain for diphtheria bacilli. I tried various combinations to increase its polychromatic properties, until one of our laboratory technicians, Richard Lundquist, suggested the idea of boiling the dye with an alkali. Heretofore, this process was used chiefly in obtaining various color compounds from methylene blue. For instance, Unna's polychrome methylene blue is prepared by boiling methylene blue with potassium carbonate. Similarly, the production of various azures is described by Tribondeau.[1] Goodpasture[2] describes a serviceable acid polychrome stain. But the polychromatic qualities obtainable from toluidin-blue seem to excel all the others in the sharpness of differentiation. Although its special usefulness lies in staining frozen sections for rapid diagnosis, it is of distinct value in a large number of routine laboratory procedures.

The preparation of the stain is as follows:

Toluidin-blue	1.0
Potassium carbonate	1.0
Distilled water	400.0

Boil solution in glass beaker until it is reduced to 300 c.c.
Cool; then add:

Toluidin-blue	2.0
Sodium chloride	3.0
Glacial acetic acid	12.0
Alcohol	15.0

Stir mixture until ingredients are completely dissolved. It is ready for use at once, and can be used over and over again. It does not precipitate and keeps indefinitely.

The following technic is employed in staining frozen sections for rapid diagnosis. A piece of tissue which is not more than 3 mm. in thickness is dropped into boiling 10 per cent formalin for about one minute. Fresh tissue without hardening may be used in many cases but the sections are more difficult to handle. The tissue is cut with the freezing microtome, and the sections

*From the Laboratory of Pathology of the Medical School of the University of Minnesota, Minneapolis, Minn.

are placed in a dish containing physiologic salt solution. The sections are quickly straightened out and carried over into a small dish of the stain where they are left for about fifteen seconds. They can be left for a much longer time without overstaining. The sections are then transferred to another dish of physiologic salt solution where they are rapidly washed for a few seconds to remove the excess of stain, and then are at once mounted in the same solution on a slide, and a coverslip applied. The section is now ready for examination. The entire process, from the time the section is cut to the completed preparation for microscopic study, need not take more than one minute in most instances.

I have found that by handling the sections in physiologic salt solution and by having approximately 1 per cent salt in the stain, the outlines of the cells and their nuclei are clearer and sharper in the resulting preparation. The acetic acid in the stain helps to accentuate the nuclei, while the alcohol aids in straightening out the section after its transference into the last salt solution.

Sections stained by this method give approximately the following microscopic pictures. The cytoplasm of epithelial cells stains a purplish blue of varying intensity depending upon the age of the cell. The younger the cell, the deeper the stain. The stratum germinativum of the skin, therefore, takes a deeper stain than the stratum corneum. The nuclei stain deep purple. Tumor cells, like those of carcinomata, stain deep purplish blue. Mitotic figures show up very distinctly, as the chromosomes stain a purplish black. Hyaline connective tissue stains pink and the intensity of the color is proportional to the degree of hyalinization present. The nuclei of the leucocytes take a very deep purple, while the cytoplasm stains only feebly bluish. The granules of the neutrophiles stain faintly bright green. The granules of the basophiles are dark purple, almost black. Plasma cells are easily identified. The cytoplasm is a light purplish blue, while the excentric nucleus appears reticular and takes a deep purple color. An achromatic zone surrounds the nucleus. Erythrocytes do not stain but appear greenish yellow. The endothelial cells lining blood vessels and lymph spaces stain light bluish purple.

The myelin sheaths of nerve fibers are bright purple, while the cytoplasm of the cells is bluish green. Cartilage stains a deep purple. Elastic fibers are brilliant green. Cells containing fat droplets present a dirty brown appearance. Because of this the zones in the lobules of a liver involved in chronic passive congestion with fatty metamorphosis stand out very prominently. In the adrenal, the cortex appears brown while the medulla is light blue.

Atherosclerosis of the aorta gives a beautiful picture. Atheromatous patches containing fat droplets are brown; elastic fibers are brilliant bluish green; and hyalinized connective tissues are pink.

Besides the staining of tissues, the examination of stools for ova and parasites is facilitated by the use of this stain. A drop of emulsified stool is mixed with a drop of the stain on a slide, and a coverslip applied. Practically all the elements in the normal stool take a purplish red color. If ova of any of the tenia be present, they stand out as regular yellowish or golden yellow bodies against a purple background. The identification of the ova thus becomes a simple matter because they appear so different from any other elements present.

But the stain is even more valuable for identifying amebæ in stools. Hitherto a correct diagnosis of ameba could be made only through the examination of a warm stool on a warming stage. With this stain, amebæ can readily be identified by taking a drop of the mucoid portion of the stool and mixing it with a small drop of the stain. The cytoplasm of the amebæ takes on a purplish blue and the outline of the cells becomes very sharp. The nucleus often shows very distinctly. Vacuoles and ingested particles become prominent. The amebæ can be recognized with the low power by their size, regularity in form, sharpness of cell outline, and the deeper bluish tinge of the cytoplasm than that of most of the other elements present. The absolute identification can then be made by means of the high power dry lens.

Diphtheria bacilli show up excellently with this stain. The smear is flooded with the stain and steamed gently for about ten to fifteen seconds. It is then washed and dried. The granules appear a deep brilliant purplish red, while the rest of the cell bodies stain a light purplish blue.

Because of the simplicity and the quite general usefulness of this stain I feel justified in reporting its formula.

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A SUBSTITUTE FOR WHITE MICE IN PNEUMOCOCCUS GROUPING

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PNEUMONIA is at all times a very prevalent disease during the winter months, and, if anything, it has been more widespread than ever this year. Especially in our army camps it has appeared in almost epidemic form.

Every effort has been made to combat this dread disease, and of no small avail has been the new treatment with the antipneumococcic serum produced by the workers at Rockefeller Institute. As this method has been published in detail in many of the current journals, it needs no repetition here.

This serum treatment depends upon the exact diagnosis of the specific type of the pneumococcus in each individual case. The method as devised by Cole[1] and his coworkers calls for a pure culture of the infecting pneumococci in the shortest possible period after the case is seen. This pure culture is necessary for the performance of the agglutination and precipitin tests with the known immune serums of Types I, II, III, and the consequent determination of the type of organism causing the infection. Owing to the high susceptibility of white mice to the pneumococcus, this purpose was found to be best and most quickly attained by the intraperitoneal injection of a small amount of sputum into one of these animals. A pure culture can be obtained in this way in from six to sixteen hours.

Because of the unusually large numbers of cases of pneumonia this winter, and because of the comparative newness of the method, breeders of white mice have not only raised the price of their animals to a ridiculous figure, but have been unable to adequately supply the demand.

To meet this emergency O. T. Avery[2] devised a quick method for grouping the pneumococcus which was independent of the use of white mice. However, he recommends the method only as an emergency, and states that it is not as reliable as the mouse method.

Being unable to procure the white mice in sufficient quantities for our work, we decided to try the common brown house mouse. For purposes of comparison of their relative susceptibility, white and brown mice were injected in duplicate with similar amounts of the same suspension of sputum. In every case the tissues of the wild mouse proved to be more susceptible to invasion by the pneumococcus as measured by the time of death and the relative number of pneumococci in the peritoneal exudate at a given time after the injection. In every case the agglutination and precipitin reactions were just as clear cut with the peritoneal washings of the brown mouse as with the white mouse.

The ordinary brown wild mouse can be obtained anywhere and at any time for the cost of trapping. We used for this purpose the small wire cage trap that can be purchased for twenty-five to thirty-five cents at almost any hardware store. A half dozen of these traps distributed among a few friends will quickly supply sufficient animals for a day's work and the supply is almost inexhaustible.

The wild mouse is more difficult to handle than his white brother, as he fights and bites as the little fiend he is. However this difficulty is easily overcome by wearing a pair of heavy leather gloves.

We report our experiences in this work in the hope that it may help others as they have certainly helped us in the present dearth of white mice.

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EDITORIALS

Functional Heart Tests

THE application of polysphygmographic, and more particularly of cardiographic, technic in the clinical diagnosis of cardiac disease has served to reveal the exact nature of many conditions which previously, in our ignorance, were classified loosely as functional cardiac disorders. No other physical sign of disturbed heart action could be detected than an alteration in rate or an irregularity in rhythm: no bruit or hypertrophy could be made out, although subjective symptoms of a decidedly alarming character might be prominent. The discovery of heart block, of auricular fibrillation and flutter, and more recently of the various so-called bundle lesions, has opened out an entirely new field in cardiac medicine and therapeutics. But even when every refinement of diagnosis is applied, there yet remain many cases in which there is perfectly evident cardiac embarrassment with no demonstrable physical sign of unphysiologic heart action. Irritable heart and so-called soldier's heart are examples of such cases.

The problem which most pressingly calls for further investigation at present

is, therefore, the development of methods by which the functional capacity of the heart can be gauged. Experimental investigation by Starling and his pupils with heart-lung preparations, in which the work by the heart may be accurately measured, had shown that the dominating factor is the venous inflow. If this remains constant, neither a great acceleration in heart rate nor an increase in arterial blood pressure can in itself alter the outflow. But the extent to which alterations in venous inflow may correspondingly alter the arterial discharge is so considerable as to permit the conclusion to be drawn that the reserve power of the heart muscle is equal to, if not greater, than the power which the heart actually expends on the performance of its ordinary pumping action. It is this reserve power which constitutes the factor of safety of the heart, and which may become narrowed down without any evident signs of cardiac weakness until some stress is thrown on the organ.

To test the extent of this factor of safety of the heart is the object of some recent investigations in the cardiac clinics. The adoption of suitable methods for the test involves two questions: first, how we are to gauge the heart's reaction, and secondly, how we are to subject the organ to approximately measurable increasing amounts of extra strain. Experience has shown that the first object is satisfactorily fulfilled by measuring the heart rate (pulse) and the systolic blood pressure, and that the second is best applied by graded muscular exercise.

Elsewhere in this JOURNAL will be found an explanation of the probable nature of the mechanism by which the cardiovascular system responds to increasing effort on the part of the somatic musculature, but at present we need concern ourselves merely with the readily measurable changes by which the response can be gauged. Rapport,[1] Barringer,[2] and Cotton, Rapport and Lewis,[3] have shown that the pulse rate and systolic blood pressure taken at frequent intervals immediately after the exercise is discontinued, furnish the most practical guides. The pulse rate may be taken either by the ordinary, palpatory, method (Barringer) or by applying to the opposite arm to that from which blood pressure is being measured a blood-pressure armlet connected with an Erlanger capsule and a polygraph; the tubing leading to the polygraph is disconnected from the capsule during the exercise, but is immediately attached when this is ended. The blood pressure is taken by auscultatory or palpatory methods, the cuff being left in position on the arm during the exercise.

It has been found that the determining factor in the heart's response does not depend on the particular group of contracting muscles, but on the actual amount of work performed (i. e., the foot pounds of work). Lifting dumb bells, walking a certain distance, or climbing stairs at a certain rate are all equally suitable methods of investigation. The most practical method would appear to be to "lift twenty-pound dumb-bells from the floor to the full stretch of the arms above the head, swinging them in one motion up and in one motion down, the complete movement occupying two, three, four or more seconds (guided by a metronome)." The movement is repeated from seven to sixty times, and its rate is governed according to the capacity of the subject, the limit being taken as the exercise that is enough to induce distress of breathing

and some fatigue. Each individual repeats the exercise on different days, and curves averaging the results obtained at each test are taken as characteristic for that individual.

Both pulse rate and blood pressure are of course increased during the exercise, and it is with regard to the immediate after-effect that the tests are concerned. Barringer states that in normal persons both pulse and pressure rapidly return to normal when the first observations are taken within 30 seconds after the work, unless an amount of work has been performed that is in excess of the heart's capacity, in which case the blood pressure does not reach its maximal height until later than 30 seconds after the work is discontinued, although meanwhile the pulse rate has returned almost to normal. According to this author the delayed rise in blood pressure always occurs after a certain amount of work (measured in foot pounds), and it does not vary in a given individual from day to day and is independent of the muscle group performing the work. But, whereas, in normal persons several thousand foot pounds of work can be done without the delayed rise, a few hundred foot pounds will cause it to appear in a cardiac case. Improvement in the heart's action is evidenced by finding that the amount of work which can be performed before a delayed rise ensues becomes greater and greater. In brief, then, Barringer believes that the "delayed rise in systolic pressure indicates that the preceding work has exceeded the limit of the heart's reserve power."

The results of Rapport, Cotton and Lewis do not entirely agree with those of Barringer. By taking the pressure measurements at more frequent intervals (the first one being taken from 3 to 10 seconds after the exercise), these authors have found that the blood pressure is usually only a little above the normal immediately after the exercise, but that it then invariably mounts to a certain level which is more or less proportional to the amount of the preceding exercise, and which is reached in from 2 to 60 seconds after the discontinuance of the work. The pressure thereafter gradually declines, to reach the normal in from 1 to 4½ minutes. In general the curves of blood pressure are identical in form for normal and cardiac cases. Such differences as exist are quantitative and not qualitative. Thus, untrained, normal subjects, by lifting twenty-pound bells forty to sixty times in double the number of seconds will usually cause the pressure to rise to about 160 mm. Hg. and the pulse to about 170 per minute. A cardiac case will show equal responses after much less work, or, if the work be the same, both pulse and pressure will rise much higher and take a much longer time to return to normal. These investigators, in contradiction to Barringer, find, however, that the summit of the blood pressure curve is not delayed.

It does not greatly concern us at present as to which of the investigators has most accurately described the essential characteristics of the responses. The important thing is that another method for testing the efficiency of the cardiovascular mechanism should be available, and one which requires no such high degree of technical skill as the polygraph and electrocardiograph. Even counting of the pulse alone following exercise supplies important information. This was shown some years ago by Pembrey and Todd.[4, 5] These authors found that

there is a marked difference in the time required, after a short bout of strenuous exercise (running up and down stairs for thirty seconds), for the pulse to return to its normal rate in athletically trained as compared with untrained men. Immediately after the exercise in the former group the rate was about 120, and it had become normal again five minutes later. In untrained men, on the other hand, the rate increased to about 160, and was still about 96 five minutes later. Meakins and Sanson[6] have found this method useful in the clinic. Any type of simple exercise can be adopted, but it must always be of the same degree so that comparison of the reactions of different individuals may be possible. Marching a certain distance at quick time followed by stair climbing (eighteen feet) furnished the exercise. Before the exercise a sphygmograph was attached to the wrist, with the patient sitting, and a tracing taken from which the pulse rate was counted during six-second periods. The sphygmograph was disconnected but the wristlet left in position during the exercise, and immediately after it the patient was again made to sit and the sphygmograph reapplied. The important point in the technic is that the pulse-counting can be done accurately for short periods of time (six seconds) immediately following the exercise. The counting was continued until the rate per minute, as estimated from six-second periods on the tracing, had returned to normal. It was found in cases of irritable heart, not only that the pulse rate was greater both immediately before and after the exercise, but—and most significantly—it took much longer than usual for the rate to regain the normal after discontinuance of the exercise. The palpitation experienced by the cardiac cases was in direct proportion to the length of time the pulse rate took to return to normal. In general the cases in which the pulse rate returned to normal within one minute had little or no cardiac distress. The importance of the method rests in its simplicity, as the patient can be observed readily from day to day and the effect of graduated exercise on the heart's efficiency properly studied.

The investigations are such as to indicate that simple quantitative tests applied following exercise are of decided value in testing the functional capacity of the heart. It would of course be desirable that all investigators should adopt uniform standards, not only for the exact type of exercise employed, but also for the methods used and the frequency of measurement.

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—*J. J. R. M.*

Colonel George E. Bushnell, Medical Corps

MEMBERS of the medical profession of this country are all familiar with the names of brethren famous in medical schools in large cities and with those prominent in medical literature. Few physicians knew that the best and most learned authority on pulmonary tuberculosis was to be found in the Medical Corps of the Army.

About a year ago when the United States entered the great war for liberty, reports were reaching us that pulmonary tuberculosis was widespread among the French troops and alarming among the civilian population of France. Would our army face similar danger was at once the thought of those interested in this disease.

The Surgeon-General was fortunate in having at hand an adviser almost unknown to the general medical profession but known to them today as a master in this disease, Colonel George E. Bushnell. For fourteen years at the army tuberculosis institution at Fort Bayard, New Mexico, Bushnell had been studying and investigating in a most scholarly manner the field of tuberculosis. There was certainly no want of preparedness in this field when war came.

In June, 1917, a paper by Bushnell,[1] entitled "Tuberculosis in the Military Service," appeared in *The Military Surgeon*. This paper was recently described by Sewall[2] as "replete with learning and analytical acumen." Revealing profound thought and study, it has furnished the knowledge and counsel by which upwards of a million men have been examined by members of the Medical Reserve Corps constituting Tuberculosis Boards.

Bushnell, a graduate in arts and in medicine of Yale University, has been in the Medical Corps since 1881. In 1901, while on duty in the Surgeon-General's office, he developed pulmonary tuberculosis and spent a short time at Asheville, N. C. In 1902 he was placed on duty as surgeon at Fort Logan, Colorado, and the following year assumed command at Fort Bayard. From his colleague, Colonel Bruns, we learn that while at Fort Bayard, Bushnell introduced the open air and rest treatment for tuberculosis, practically rebuilt the entire hospital, planned all the landscape gardening, raised a great many of the trees from seed, and worked incessantly, developing every phase of the institution.

A great service was rendered to the country generally when a few years ago Bushnell[3] published an important contribution to counter the growing public fear of tubercle infection in adult life. A recent publication[4] by the same writer shows how exaggerated too were the first reports of tuberculosis that reached us from France. The clinical investigations of the healthy, as well as the diseased, led Bushnell[5, 6] to the correct understanding and interpretation of marginal rales, sternal rales, etc. These were signs that had long confused many physicians here and abroad as can well be recognized by consulting the literature.

The country has been most fortunate in having Bushnell called to head the Department of Internal Medicine in the Surgeon-Generals' office at the outbreak of the war, and the medical men who have been connected with this department have met with most considerate and kindly treatment and have

recognized the guiding hand of a master. Though he retired from active service on account of age, Sept. 10, 1917, it is a great satisfaction to know that Colonel Bushnell is still in charge of this most important work.

It must be a wonderful gratification to this medical officer that after a life of preparation for such a role the country's call came for the exercise and help of his many natural and acquired talents.

"Non sibi sed toti."

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—*G. B. W.*

Further Researches on the Physiology of the Adrenals

A YEAR ago an editorial in these pages reviewed the work of Stewart and Rogoff on the physiology of the adrenals. Using the denervated iris, which responds with dilation to very small doses of adrenalin as a test for epinephrin in the blood, these authors were unable to confirm the general impression which recent work had created, that the adrenals, besides continually secreting some epinephrin into the blood, act as auxiliary organs to the symphathetic system and in times of stress pour out an increased amount of epinephrin into the blood, producing thereby an increase in the blood pressure and muscular power, and decreasing the clotting time of the blood. They have since then published a number of researches which add much to our knowledge of the adrenals, and also throw additional doubt on the validity of the current theories of adrenal function.

Stewart and Rogoff have sought for the various factors which are alleged to influence the secretion of epinephrin by collecting the blood flowing from the adrenal glands in a pocket made from the lower portion of the vena cava and testing for epinephrin by the classic methods, viz: the inhibition of the movements of the rabbit's intestine and the contraction of the rabbit's uterus. They find that the rate of secretion of epinephrin is, under ordinary conditions, very constant, and is not influenced by painful stimulation of large nerves or by asphyxia. The concentration in which it occurs in the blood is, however, found to be inversely proportional to the amount of blood flowing through the adrenals, and for this reason they point out that any calculation of the amount of epinephrin secreted must not be based on the concentration found in a sample of blood issuing from the adrenal vein without taking into consideration the amount of blood flowing through the adrenals.

Cats in which the nerve supply to the adrenal glands had been severed remained well and apparently normal for as long as five weeks, when they were purposely sacrificed. The epinephrin reactions obtained on the iris or on the blood pressure showed an absence of the secretion. The authors believe, therefore, that epinephrin is not indispensable to the body, and moreover that its secretion is entirely under nervous control.

They have also investigated the alleged relationship of the secretion of epinephrin to certain hyperglycemias, and in cats in which the secretion of epinephrin was rendered impossible through section of the splanchnic on one side and the removal of the adrenal on the other, they have failed to find evidence that hyperglycemia produced experimentally by anesthesia, asphyxia, or by alleged emotional stress, is less than in normal animals.

The normal physiologic function of epinephrin, if it has any, is apparently still in the dark. The simple and highly active hormone which was thought to regulate and augment so many and such varied physiological processes, becomes again a physiologic problem.

For further details see paper by Rogoff in the January issue of this JOURNAL.

—*R. G. P.*

Acapnia and Shock

SHOCK is a condition of low vitality bordering on death. Of its many symptoms some are primary, in the sense that they are dependent upon the initial disturbance causing the condition, and some are secondary, being merely the inevitable consequence of the physiologic depression. The first step in the investigation of the cause of shock hinges on a correct differentiation between the two classes of symptoms. Low blood pressure, nerve cell fatigue, acidosis, depletion of the alkaline reserve, etc., to name only a few of the prominent symptoms, has each in turn been exploited as the primary cause of shock, but although all of these symptoms may be causative in a certain sense, no one can be pointed to as occupying so conspicuous a position in this regard that remedial means, capable of removing it thereby also cause recovery from the shock itself. The blood pressure, always low, may be temporarily raised and the patient rally so long as the restoration lasts, or the administration of alkali, as bicarbonate may, as Cannon reports, produce definite improvement; but even so, this does not indicate that the cause of shock is the low blood pressure or the bicarbonate deficiency. When we maintain life for sometime longer in an animal moribund from starvation by placing it in an incubator so as to prevent fall in body temperature, we do not thereby prove that the fall of temperature is the cause of death. In a complicated clinical condition like shock, where there are many conspicuous subjective symptoms, each new objective symptom, as it is discovered by the applictaion of more refined means of diagnosis, is certain to be heralded as the progenitor of all the other symptoms; it is pronounced the cause of shock.

And there is another aspect of the problem which one must not pass by--

namely, the failure to explain why it is that the particular symptom that is supposed to be "the cause" should not infrequently be prominent in other diseases in which, however, "shock" is absent. An animal with the spinal cord severed above the level at which the vasoconstrictor fibers leave, and in which therefore the blood pressure is extremely low, shows no symptom of shock in the portions of the body anterior to the lesion. Neither is there shock when the alkaline reserve of the blood is greatly depleted, as in severe nephritis or diabetes. Other symptoms, it is true, develop but they are not those of shock.

These preliminary remarks will serve to introduce the main subject of this editorial—the relationship of acapnia to shock. Some ten years ago Yandell Henderson announced that shock is closely dependent upon a deficiency in the amount of carbonic acid in the blood, usually brought about by excessive removal of CO_2 by way of the lungs; a "blowing off" of the volatile acid of the blood. It was claimed that, in the shock following stimulation of sensory nerves, the "blowing off" occurs because of the hyperpnea thereby induced, whilst in abdominal operations diffusion of CO_2 from the exposed peritoneum into the air is an important factor. The proof offered in support of these claims was, partly, that the blood analyzed by the Haldane-Barcroft apparatus contained a low per cent of CO_2, and partly, that the shock could be prevented during excessive pulmonary ventilation by maintaining a fair percentage of CO_2 in the inspired air, or in abdominal operations by taking precautions to prevent diffusion of CO_2 from the exposed viscera. The investigations showed that a low content of CO_2 in the blood is at least one of the prominent accompaniments of certain forms of experimental shock, but that it is not the cause of the condition as met with in man, was amply shown by other observers, on the one hand, by the fact that shock may develop in cases where the breathing instead of being exaggerated is from the beginning infrequent and shallow; and on the other, by the failure to remedy the shock by causing the patient to respire in a CO_2-rich atmosphere. Temporary improvement accompanied by increased respiration might result from this treatment, an observation which is no more remarkable than that the respirations in many cases of Cheyne-Stokes (periodic) breathing should become regular when the patient is made to respire in a CO_2-rich atmosphere. In such a case it is recognized that we do no more than temporarily excite the depressed respiratory center; we do not remove the cause of the depression; we only alleviate one symptom resulting from it.

For these and many other reasons the acapnia theory of shock was dignified some eight years ago by an apparently adequate burial, but from the remains has now sprung into being a new, reincarnated acapnia theory, with its birthplace in the pages of one of our most excellent scientific journals, the *Journal of Biological Chemistry*.[1] Surely the reincarnated infant is blessed by the environment of its birth.

In its revised form, acapnia is defined as a deficiency not merely in the amount of CO_2 in the blood, but also in the capacity of the blood to absorb this gas. Under the old definition it was evidently assumed that there might be a deficiency of CO_2 without an accompanying curtailment of CO_2-binding power of the blood, and acknowledgment is made to Van Slyke and his collaborators

for "the conception" of the alkaline reserve which has prompted the new definition. In so far as acapnia acts as a causative factor in shock, it would appear from the new definition that it is the depletion of alkaline reserve rather than the diminution in actual CO_2 content that is important. It is, however, somewhat difficult to see from their papers what the authors really mean and their results do not contribute any facts which were not perfectly self-evident in the previous papers of Henderson himself.

Briefly stated the experimental procedure adopted in the present research consisted in removing arterial blood from dogs subjected to various conditions that we know to cause increased or diminished excretion of CO_2 by way of the lungs, and determining in this blood first, the actual CO_2 content, and secondly, the CO_2 content after exposing the blood in a suitable apparatus to an atmosphere containing a known percentage of CO_2. Comparison of the results of the two determinations indicates to what extent the CO_2 absorbing power of the blood is satisfied, whereas, those of the second method taken alone give us the same information as the now well-known Van Slyke method—namely, the reserve alkalinity or, as the authors call it, the CO_2 capacity of the blood. The alveolar air was also collected for analysis in a few of the experiments by the uncertain Higgins-Plesch method. In the first series of experiments, the effect of raising the CO_2 of the blood was investigated when the CO_2 tension of the alveolar air was raised either by decreasing the extent of pulmonary ventilation through depression of the respiratory center by means of morphine, or by causing the animals to respire into a bag containing 5 or 6 per cent CO_2. The increase in the CO_2 of the alveolar air promptly caused an increase in the CO_2 content of the blood, followed later by an increase in CO_2 capacity. In only one of the experiments, and in this case the rectal temperature was very low, did the capacity come up to the actual amount even after several hours. In other words, there was constantly present in the blood a ratio between H_2CO_3 and $NaHCO_3$ which was distinctly greater than $\frac{1}{20}$, under which conditions, as R. W. Scott[2] has shown in this laboratory there is a readily recognizable increase in the H-ion concentration of the arterial blood. In one experiment (No. 10) the respired air contained a very high percentage (20) of CO_2, so that the total CO_2 of the blood rose to 80 and the CO_2 capacity to 74; and when the breathing bag was removed and air again respired, the animal underwent apnea, because, it is said, "the alkali content of the blood had been raised so high that, when the abnormally high pressure of CO_2 was withdrawn, the respiratory center lacked an adequate stimulus."

These experiments bring out absolutely nothing new. Zuntz many years ago showed that, when CO_2 is added to blood the bicarbonate content is increased, and more recently R. W. Scott[2] by comparison of the H-ion concentration and the CO_2 content demonstrated that, when the total CO_2 content of the blood is raised by breathing CO_2-rich air, the ratio between H_2CO_3 and $NaHCO_3$ becomes raised but the increase in $NaHCO_3$ lags behind that of H_2CO_3. Under the conditions of Henderson's and Haggard's experiments, therefore, there must have been an elevation in the H-ion concentration of the blood—a carbon-dioxide acidosis.

In the next paper experiments are described in which the CO_2 content of the blood was experimentally lowered, and it is shown that the CO_2 capacity also became depressed. To lower the CO_2, hyperventilation was induced by administering ether in such a way that there was constant hyperpnea, which "washed out" or "blew off" the CO_2 of the blood. If this was allowed to go on until the CO_2 content and capacity had fallen to about 33, irrecoverable shock occurred. When the ether was properly given, so as to produce deep anesthesia without preliminary hyperpnea, no decrease in the CO_2 content or capacity, rather an increase, was observed, but no shock. Nor was there any shock produced by faulty etherization when the respired air contained CO_2 in sufficient percentage to maintain the normal level of CO_2 content and capacity in the blood. Even when a considerable depression had already occurred, the normal level could be restored by breathing a CO_2-rich atmosphere, provided the depression had not reached to from 33 to 36 per cent CO_2.

These experiments again are merely confirmatory of those of Milroy,[3] who showed that a definite fall in hydrogen ion of the blood resulted from forced ventilation, which, however, was not the case when the air was rich in CO_2. It necessarily follows, as has been explained above, that there must be a fall in $NaHCO_3$ under these conditions. The point which the authors would particularly emphasize is that ether *per se* does not produce "acidosis" by some obscure effect on metabolism. Why this fact should be given more prominence than that a condition of alkalosis is established, is difficult to comprehend. Is it meant by the authors that the process by which the alkali leaves the blood is dangerous or is it the actual decrease of the absolute amount of alkali in the blood? It is much more likely that the relative excess of $NaHCO_3$ over H_2CO_3, causing depression in C_H, is in part responsible for the shock-like symptoms.

Papers are also contributed to show the already well-known fact that excessive artificial respiration induced in other ways lowers the CO_2 content of the blood and of course the CO_2 capacity, there being at the same time a fall in arterial pressure with the reflexes at first hyperactive and later suppressed. When the hyperventilation is conducted with air containing 6-8 per cent CO_2, on the other hand, the blood CO_2 does not fall so long as this air is breathed. Only one experiment of this last-mentioned type is reported, however, and in this it is shown that, although the blood pressure, as would be expected, rises during the forced ventilation, it falls immediately thereafter. The authors attribute the fall to chloretone, whereas the fall in the other experiment, also on chloretonized animals, is attributed to shock. Why the difference?

It is natural to conclude that since excessive hyperventilation of the lungs by artificial respiration can in itself depress the blood pressure, so also will the hyperpnea produced by overactivity of the respiratory center as a consequence of stimulation of sensory nerves. The sensory nerves stimulated were those of the peritoneum around the duodenum in animals anesthetized by morphine and believed therefore not to experience any pain. The usual results were of course obtained. If the air breathed contained a high percentage of CO_2, however, the blood pressure remained normal for a longer time (in two experiments), although in one of the experiments (No. 1) a fatal condition of shock, with con-

gested intestines, etc., supervened (there was, however, some hemorrhage in this animal).

In short, the whole series of papers is a repetition of work already published by Henderson himself, the only addition being that the alkaline reserve or, as the authors prefer to term it, the total CO_2 capacity of the blood is examined with results that are inevitable from the accurately controlled work of other observers. That depression in the CO_2 content and therefore the CO_2 capacity of blood occurs in certain experimental conditions simulating shock as met with in the clinic, and that it does not occur when this depression is artificially prevented is exactly the same evidence as was advanced some years ago in favor of the theory that the acapnia is a causative factor in shock. There is no reply to the many objections that have been raised to the hypothesis, for example, why it should be that the blood of patients in shock often contains a practically normal CO_2-content (Rendel Short), why shock may occur after forced artificial respiration in animals even when the CO_2 content of the blood is kept high and conversely, why it should often be absent when the CO_2 of the blood is persistently very low, why it often occurs early in patients (½ hour) in whom very little hyperpnea is present, and in patients anesthetized by a face mask (Clover's) which would cause them to rebreathe considerable percentages of CO_2, and so on.

From the biochemical standpoint, the conclusion may, however, be drawn that excessive depletion of free CO_2 of the blood will cause a temporary condition of alkalosis, because it will take some time before the alkaline reserves will lower themselves to the new level. Whether this in itself can bring on a condition of shock is doubtful, for we have repeatedly injected enormous quantities of alkali, sufficient to lower the H-ion concentration for considerable periods of time, without any shocklike symptoms supervening. During this period of lowered H-ion concentration it is natural to expect that there should be a depression of many physiologic functions and particularly of the nerve centers whose tone or activity is known to be highly sensitive to H-ion concentration. The condition, however, is not shock. The temporary alkalosis can be remedied by adding acid to the blood, and probably the most convenient way to do this is by inhalation of CO_2-rich air, although exactly the same results are attained by injecting faintly acidified saline solution intravenously.

We would sum up by saying that this new work of Henderson and Haggard is a repetition of experiments of the same nature as those by which it was attempted to show that acapnia is an important causative factor in shock. No new fact is contributed to controvert the many objections that have been raised against the theory.

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—*J. J. R. M.*